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# A Dynamic Model of the Kinetics of the Cell Cycle in Higher Eukaryotes.

Harold Joseph Toups

*Louisiana State University and Agricultural & Mechanical College*

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A DYNAMIC MODEL OF THE KINETICS  
OF THE CELL CYCLE IN HIGHER EUKARYOTES

A Dissertation

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Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy

in

The Department of Chemical Engineering

by

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August, 1973

## DEDICATION

To:

My wife, Margaret and daughter, Lauri for everything - especially  
for hugs, kisses, and weiner sandwiches.

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## FOREWORD

The involvement of engineers, particularly chemical engineers, in the fields of biomedicine, biochemistry, biomathematics, and ecology can be justified by the unique combination of skills they possess. Training in advanced computational methods, systems analysis, and chemistry enable the chemical engineer to tackle problems in a successful fashion in these newer fields.

Such efforts are interdisciplinary by nature and therefore require the engineer to possess special communicative skills in order to be understood as well as to understand. This dissertation has been undertaken with an effort to utilize these skills.

## TABLE OF CONTENTS

	page
DEDICATION.....	ii
ACKNOWLEDGMENT.....	iii
FOREWORD.....	iv
LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
ABSTRACT.....	xi
 CHAPTER	
I.    INTRODUCTION.....	1
A.    Cell Theory.....	1
1.    Cell Chemistry.....	2
2.    Mechanisms of Synthesis.....	5
a)    Replication.....	6
b)    Transcription.....	7
c)    Translation.....	7
3.    Cell Growth and the Cell Cycle.....	10
a)    Description of the Cell Cycle.....	10
b)    Single Cell and Synchronous Culture Methods.....	12
4.    Dynamics of Growth.....	13
B.    Cell Models.....	15
1.    Continuous Analysis.....	16
2.    Automata Theory.....	25
3.    Logical Control Models.....	26



	page
CHAPTER	
C. Summary.....	28
II. MODEL DEVELOPMENT.....	33
A. Derivation of Higher Eukaryote Cell Model.....	33
1. System Description.....	34
2. Material Balances.....	41
3. Initial and Final Conditions.....	44
B. Development of Dimensionless Cell Equations...	45
1. Material Balances.....	45
2. Initial and Final Conditions.....	47
3. Messenger RNA Decay.....	49
C. Establishment of an Operational Cell Growth System.....	49
1. Numerical Schemes.....	50
a) Integration Scheme.....	51
b) Search Technique.....	53
2. Demonstration of the Existence of a Single, Physically Meaningful Solution.....	54
III. RESULTS.....	60
A. Normal Growth Studies.....	60
1. DNA.....	61
2. RNA Fractions.....	62
3. Protein.....	68
4. Cell Volume.....	70
B. Deviations from Normal Growth.....	73

	page
CHAPTER	
1. Blockage of DNA Synthesis.....	73
2. Stability of Cell Model Subjected to Stress.....	78
3. Messenger RNA and Cancer.....	87
C. Initiation of DNA Synthesis.....	93
D. Summary.....	96
IV. CONCLUSIONS AND RECOMMENDATIONS.....	99
APPENDIX	
A. NOMENCLATURE.....	102
B. GLOSSARY OF CELL BIOLOGY TERMS.....	106
C. GLOSSARY OF CELL MODELING TERMS.....	109
D. ORDER OF MAGNITUDE ANALYSIS.....	110
VITA.....	116

# LIST OF TABLES

TABLE		page
I-1	Mathematical Models of Cells.....	17
II-1	Eukaryotic Cell System Components and Their Functions.....	35
II-2	Schematic of Principle Reaction Paths in Eukaryotic Cells.....	36
II-3	Revised Set of Reaction Mechanisms.....	38
II-4	Resultant Material Balance Equations.....	43
II-5	Normalized Material Balance Equations.....	48
II-6	Comparison of the Results of a Bounded Search over a Range of Rate Constants.....	58
III-1	Results of a Statistical Study on the Relation of Protein Synthesis to the Initiation of DNA Synthesis.....	95

## LIST OF FIGURES

FIGURE		page
I-1	The Cell Cycle in Most Higher Eukaryotes.....	11
I-2	Model System of Yeisley and Pollard.....	20
II-1	Logic Flow Diagram for Establishing a Functional Cell Model.....	52
III-1	DNA in Mouse Fibroblasts during Normal Growth.....	63
III-2	Ribosomal RNA in HeLa S3 Cells during Normal Growth....	65
III-3	Messenger RNA in HeLa S3 Cells during Normal Growth....	66
III-4	Transfer RNA in HeLa S3 Cells during Normal Growth.....	67
III-5	Protein in Mouse Fibroblasts during Normal Growth.....	69
III-6	Volume Growth Rate in Chinese Hamster Ovary Cells during Normal Growth.....	72
III-7	Ribosomal RNA in HeLa S3 Cells Subjected to a Block in DNA Synthesis.....	75
III-8	Messenger RNA in HeLa S3 Cells Subjected to a Block in DNA Synthesis.....	76
III-9	Transfer RNA in HeLa S3 Cells Subjected to a Block in DNA Synthesis.....	77
III-10	Logic Flow Diagram for Stability Study.....	80
III-11	Time Response of Free mRNA in Mouse Fibroblasts to Change in Free mRNA.....	82
III-12	Time Response of Protein in Mouse Fibroblasts to Change in Free mRNA.....	83
III-13	Time Response of DNA in Mouse Fibroblasts to Change in DNA.....	84
III-14	Time Response of mRNA in Mouse Fibroblasts to Change in DNA.....	85

FIGURE		page
III-15	Time Response of Protein in Mouse Fibroblasts to Change in DNA.....	86
III-16	DNA Response to Change in Free mRNA in Cells with Rapid Decay of mRNA.....	89
III-17	Protein Response to Change in Free mRNA in Cells with Rapid Decay of mRNA.....	90
III-18	Free mRNA Response to Change in Free mRNA in Cells with Rapid Decay of mRNA.....	91

## ABSTRACT

A simplified model of the metabolic and replicative processes indigenous to the cell cycle of higher eukaryotes has been developed. A series of nonlinear differential equations is used to represent changes in various biochemical species within a cell undergoing isothermal growth in relatively rich media. Changes of variable have been utilized in solving the system equations in a generalized fashion. Conditional logic has been used to trigger the initiation and cessation of DNA synthesis, and cell volume was rightly considered as a variable quantity. The model correctly predicts changes in the chemical constituents of several eukaryotic cell types.

An analysis of abnormal growth has been used to test and refine current hypotheses concerning cellular control of biochemical reaction rates and metabolic upsets as carcinogenic stimuli. This analysis was performed through simulation of:

- (i) the blockage of DNA synthesis,
- (ii) the imposition of stresses, and their effects on cell stability, and
- (iii) the stabilization of cell growth by messenger RNA decay.

A statistical study, based on a variety of cell lines, utilized the model to corroborate evidence of a relationship between cell protein quantity and DNA synthesis initiation.

This general model should be reliable for synthesis pattern prediction in higher eukaryotic cells that aren't markedly different from those described by the model system.

## CHAPTER I

### INTRODUCTION

Historically, research in the life sciences is based upon an experimental approach. Descriptive analysis is only recently being complimented by quantitative mathematical study in an effort to describe and integrate phenomena into the framework of a total system. However, even a single cell - a fundamental biological unit - is a maze of structural and functional complexity. This research is an effort to model in a relatively simple form, the growth and replication processes which occur during the cell cycle of higher eukaryotes\* (the most highly evolved cells). Because of the wealth of experimental data available, until recently bacteria and lower organisms have been studied almost exclusively. But mammalian cells and other eukaryotes provide a potentially richer area for research since man's concern for the human system is paramount.

The interdisciplinary nature of the research calls for some background in cell theory. This will preceed the discussion of previous modeling efforts.

#### A. Cell Theory

Just as in physics where the atom is acknowledged with special importance as a fundamental unit, so biologists treat the cell. The quite familiar idea that the cell is the basic unit of life embodies

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\*A glossary of pertinent biological terms will be found in Appendix B.

what is known as the cell theory. Arthur Hughes' A History of Cytology [1] attributes the first formulation of the cell theory to two German scientists, M. S. Schleiden and Theodor Schwann, in 1838. Further discoveries and experiments have led to a modern interpretation with three principle generalizations. The first is that life exists only in cells; an organism's existence and activity depends on cells. Secondly, cells come only from pre-existing cells; the Hughes' account cites Rudolph Virchow as the author of this generalization. The third idea is that of complementarity, i.e., there is a distinct relation between structure and function. These primary ideas form a basis for the modern definition of a cell: the cell is the smallest living unit capable of autonomous growth and reproduction using food substances chemically different from itself.

For purposes of this study, background in cell chemistry, mechanisms of synthesis, the cell cycle, and dynamics of cellular growth prove helpful and sufficient.

### 1. Cell Chemistry

As early as the 1830's when the cell theory was being formulated, food chemists recognized that the principle compounds which comprised the bulk of cellular material were lipids, carbohydrates, and proteins. Cells contain on the average 75-85% water, 10-20% protein, 2-3% lipids, 1% carbohydrate, 1% nucleic acids, 1% salt, and small amounts of various metals [2].

Lipids are commonly glycerol based compounds composed of fatty acids and, hence, are only sparingly soluble in water. Lipids tend



to aggregate in an aqueous media and form surfaces such as the membranes that hold the cell together.

Carbohydrates are compounds composed of carbon, hydrogen, and oxygen in ratios of 1:2:1, respectively. These include saccharides such as sugars and starches found in cells as food and stored matter-energy, and as the component structural materials of nucleic acids and related substances.

Protein materials constitute the greatest percentage of the dry weight of cellular components. They are composed of carbon, hydrogen, oxygen, nitrogen, usually sulfur, and sometimes phosphorus in the form of amino acids - the so-called building blocks of proteins. Although there are many possible amino acids, natural proteins seem to be made from a group of only 20 common ones. These may be arranged in any order and in any ratio but the individual character of a particular protein depends primarily on a particular order and ratio.

Enzymes are biocatalysts, a class of proteins produced by the cell. They function not only to catalyze reactions, but provide for their own controlled rate of synthesis by means of feedback mechanisms. As with some conventional catalysts, enzymes are quite specific in their action. The design of these protein molecules actually found in living cells is highly efficient from the point of view of information processing. Branson [3] made calculations on the possible information content of such proteins by assuming that the arrangement of amino acid residues was the key factor. He concluded that these

substances have a remarkably high information level, none of the ones studied having less than 70% of the theoretical maximum.

The relatively large amount of water present in the cell is essential from two standpoints. First, all the chemical reactions necessary to life take place in aqueous media and, secondly, water together with the salts and other materials, provides an osmotic balance for cells. In this regard, small quantities of various metals are often necessary as cofactors for biochemical reactions. Also, certain enzymes essential to the cell's chemical activities must be activated by specific metallic salts.

The most important class of substances native to living matter are the nucleic acids. Discovered in 1868 by Fredrich Miescher [1], it took nearly eighty years before science recognized that nucleic acids constitute the genetic material of living cells. Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the two key nucleic acids found in all cells [4]. Nearly all of the DNA is located in the nucleus, or controlling center of the cell. Conversely, RNA is found both in the nucleus and in the cytoplasm or remainder of the cell.

DNA and RNA molecules are very large. The molecular weights of these substances are on the order of several million [5]. Of the two, generally RNA molecules are the smaller. Both DNA and RNA are polymers composed of a skeleton of pentose and phosphate with various purines and pyrimidines attached. The pentose of DNA is deoxyribose and that of RNA is ribose. The most common purines are adenine (A) and guanine (G), and the most common pyrimidines are cytosine (C), thymine (T), and uracil (U). Thymine is indigenous to DNA and uracil to RNA. The relative amounts of A, G, C, T and U may vary from organ

to organ in a multicellular system [5], but the DNA of one cell type is the same in all cells of that type.

The dawn of the real era of molecular biology occurred with the J. P. Watson and F. H. C. Crick derivation of the structure of the fundamental DNA unit in 1953 [6]. Their model states that DNA is a long, fibrous molecule of two interwoven chains or polynucleotides in the shape of a double helix. Hydrogen bonding of the facing bases joins the two chains and asserts that the A in one spiral is always connected to T in DNA and U in RNA. G is always bonded to C in both.

It is the pattern or arrangement of these base pairs in the polynucleotide chains which affects the information-carrying capability of DNA. The basic message unit or codon is composed of not one but three adjacent bases [7]. Each codon "codes" for a specific amino acid residue in a polypeptide chain [8]. The code itself is translated without "punctuation" from a starting point to an end point in non-overlapping fashion [9]. The current definition of the gene is a collection of codons responsible for a single polypeptide. This definition is strictly an administrative one and hence may change with new discoveries.

Cell chemistry and the structure of the chemical constituents leads directly to a study of function. The mechanisms of synthesis lend meaning to this complementarity.

## 2. Mechanisms of Synthesis

The information bearing nucleic acids and proteins are the "key molecules of living systems" [10]. The basic question of how cells reproduce can be best answered in terms of these molecules, but more

specifically, however, by means of the two distinct functions of DNA, namely the heterocatalytic and the autocatalytic functions. This basic question of cellular reproduction can thus be restated in terms of these functions. First, how does DNA control or preside over the heterocatalytic synthesis of protein for cell structures necessary for growth? Secondly, how does DNA autocatalytically replicate itself so as to provide both daughter cells with an exact complement of genetic information?

#### a) Replication

Fortunately enough, the model proposed for the structure of the DNA unit also provided a convenient explanation for how it might replicate itself. Watson and Crick's own hypothesis required the two polynucleotide chains to unravel while the synthesis of new DNA took place [11]. Each strand thus provides a template against which available molecules can pair to produce two new double-stranded molecules. This semi-conservative mechanism infers that one strand of the original DNA is present in each of the descendent chains. Through the use of heavy isotope labeling and density gradient centrifugation, Meselson and Stahl in 1958 [12] demonstrated by chemical techniques this method of replication.

The replication of DNA can be viewed as a polymerization reaction catalyzed by an enzyme. In vitro synthesis of a DNA molecule in the presence of all four precursors and a template DNA was achieved by Arthur Kornberg [13] using a purified enzyme which he called DNA polymerase. Whether or not this same mechanism occurs in vivo is still a matter of heated debate, however.

## b) Transcription

An understanding of the heterocatalytic function of DNA remained for the 1960's. These are the processes which actually transform the genetic information carried by the nucleotide sequence of DNA into the amino acid sequence of the corresponding polypeptide chain.

Transcription involves not only DNA but the other basic nucleic acid, RNA. The fact that certain RNA molecules closely resemble DNA in composition is no accident since transcription involves the synthesis of RNA through the use of DNA as a template [14, 15, 16, 17].

The template hypothesis, implying a one-way transfer of genetic information from the DNA molecule to RNA to protein, was entitled by Crick [18], the Central Dogma and can be written as



The first link in the heterocatalytic "Central Dogma" chain is accomplished by messenger RNA (mRNA). DNA serves as a template for the polymerized synthesis of mRNA with encoded amino acid information as its make-up. In this way, the gene's precise nucleotide sequence is transcribed into a "messenger" - mRNA. This equivalence of base sequences was demonstrated in 1961 by Hall and Spiegelman [14].

## c) Translation

The second stage of the template hypothesis calls for a protein synthesis "factory". Cytochemical studies by Brachet, Caspersen and others [4], as well as quantitative biochemical analysis [19], suggested that a strong correlation existed between the cytoplasmic RNA content of cells and their rate of protein synthesis. At first

glance one might infer that mRNA was responsible for this, but experiments by Maaløe [20] indicated that the amount of RNA and protein per cell nucleus increased with growth rate, while the quantity of DNA per nucleus remained constant. This suggests that RNA and protein synthesis are related to each other but dissociated somewhat from DNA synthesis.

In addition to mRNA, there are two other major varieties of RNA, ribosomal RNA (rRNA), and transfer RNA (tRNA). Nearly 80% of cellular RNA is ribosomal and occurs bound to protein in particles called ribosomes. These particles are located in the cytoplasm of cells. By means of tracer studies with radioactively tagged amino acids, the ribosomes were identified as the sites of rapid amino acid uptake and initial incorporation into protein form [21, 22]. It thus became evident that the specificity in translation is a function of the mRNA and not the rRNA [23].

So far mRNA and rRNA (in ribosomes) have been discussed. However, by what means are the raw materials - amino acids - assembled in the correct sequence for the given message? Crick himself analyzed this problem and proposed the natural hypothesis that the amino acid is carried to the template by an adaptor-type molecule, and this adaptor is the part which actually combines with the RNA [18]. In its simplest form this hypothesis would require twenty adaptors, one for each amino acid.

This hypothesis has since gained full acceptance. Soon after, a 'soluble' RNA fraction which binds amino acids was discovered [24, 25, 26]. Part of this RNA, called transfer RNA (tRNA), must be capable of recognizing the messenger codon in order to be specific

for an amino acid. The means suggested for such recognition is that tRNA possesses an anti-codon or complementary triplet of nucleotides which binds, by hydrogen bonding, to the messenger bases. Another essential item which was verified, is that ribosomes possess the site or sites to which a molecule of tRNA, or an amino acid-tRNA complex (known as aminoacyl tRNA), can become attached [27, 28, 29, 30]. These aminoacyl tRNA molecules are formed by the enzyme-mediated union of the specific amino acid and particular tRNA concerned [31]. The enzymes involved are called aminoacyl tRNA synthetases. The mRNA has an affinity for another portion of the ribosome and is bound at that point [32].

Thus a complete picture of the process portrays an aminoacyl tRNA molecule held in a slot in the ribosome in such a fashion that its anti-codon and the mRNA codon (also held to the ribosome) meet by hydrogen bonding. Another aminoacyl tRNA is held nearby, bound to the second codon of the mRNA. A dipeptide is formed by bonding of the two amino acids and the first tRNA is released [29]. This process continues with the ribosome moving along the mRNA; what causes this movement is, as yet, unknown. When a particular mRNA is completed the ribosome is again available for use. The rRNA and tRNA fractions are relatively stable [33, 34], whereas bacterial mRNA is unstable [35]. Eukaryotic cellular mRNA is much more stable than bacterial mRNA because of a difference in degradative enzyme concentration [36, 37].

Thus, the "Central Dogma" represents the foundation for the relationships between the nucleic acids and protein synthesis. The results of numerous biochemical and genetic studies have forged the new field of molecular biology, a field impossible to define to the

satisfaction of all; however, how all this fits into the cycle of life is the next question which begs an answer.

### 3. Cell Growth and the Cell Cycle

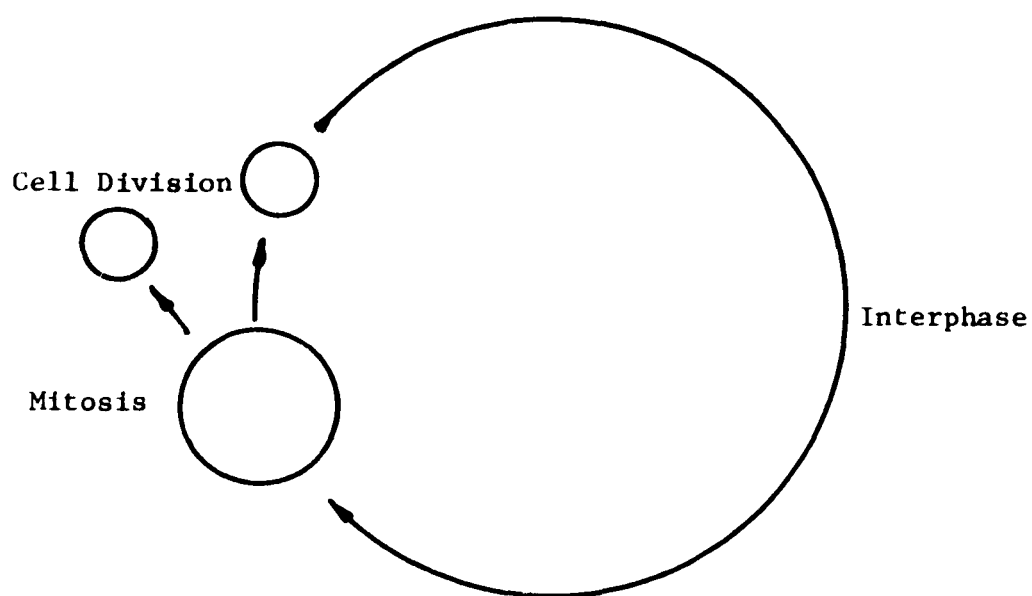
#### a) Description of the Cell Cycle

The most convenient frame of reference to use in the study of cell growth is the cell cycle, i.e., the period between the formation of a cell by division of its mother cell and the division of the cell itself into two new daughter cells. This unit of time is fundamental because it defines the life cycle of a cell.

A diagram of the cell cycle is shown in Figure I-1. This is the form taken by most higher eukaryotes. The greater portion of the cycle is interphase, the time between cell division and mitosis (the division of nuclear material). Mitosis represents only a very small fraction of the cell cycle time of most higher eukaryotes. In order to form two equal daughter cells identical to the original cell at the beginning of the cell cycle, a synthesis must occur which will double the quantity of each cell component.

Thus, characterizing the progress of cells through their life cycles are four stages of development, the nomenclature of which was first introduced by Howard and Pelc in 1953 [38]. These are: G<sub>1</sub>, gap before DNA synthesis; S, DNA synthesis; G<sub>2</sub>, gap after DNA synthesis; and M, mitosis. Actually the G<sub>2</sub> overlaps M so that in fact there is no clear demarcation between the two. Mitosis is a morphological feature and thus is in a category which differs from the synthetic events of the cell cycle. Quite commonly, the analytical methods used to define the G<sub>1</sub>, S, and G<sub>2</sub> periods do not measure the M period,





The Cell Cycle in Most Higher Eukaryotes

FIGURE I-1

but rather include it in with the others. However, since it is relatively short, this period does not always contribute significantly to these synthetic processes.

Most of the variation in generation time (cell cycle time) from one cell type to the next, takes place in the G1 period. During this period the cell prepares for DNA synthesis while producing RNA and protein. DNA synthesis is assumed to take place solely during the S period, but the uncertainty in cell measurements makes such a statement only approximately valid. The mechanism for initiation of DNA synthesis is as yet unknown. Some evidence does exist, however, which suggests a causal protein and DNA synthesis relationship [39, 40]. The G2 phase of the cell cycle is similar to the G1; though, for bacteria in rapid growth, both are practically non-existent. As previously mentioned, mitosis, for the purpose of this study, will be assumed to be of negligible duration.

#### b) Single Cell and Synchronous Culture Methods

There are essentially two means by which experimental information can be obtained on the patterns of growth and synthesis during the cell cycle. One of these is work done with single cells and observed by microscopic studies. The obvious advantage of fine detail is perhaps outweighed by the disadvantage of limitations on just what can be observed in this fashion.

Synchronous culturing has overcome this disadvantage to a great extent. Synchronous cultures are defined as those in which the cell cycles of all the individual cells have been brought into phase. This makes possible bulk biochemical measurements that are not

possible with single cells. The one big problem is in establishing "the high degree of synchrony" necessary for accurate measurements. Also, once established it only takes a few cycles to make the cell culture become asynchronous again [41]. Perhaps the most important attendant fault here is the fact that not only are finer details of synthetic patterns obscured, but the method of synchronization may have an effect, as well, on the patterns that are observed.

The two principle methods of synchronization are selection synchrony and induction synchrony. Selection synchrony simply involves selecting cells in the same position in the cell cycle from a culture of normal, unsynchronized cells and growing them as a new culture. Induction synchrony requires that unsynchronized cells be treated in some way (either chemically or thermally) so as to induce them into synchrony.

Various other analytical procedures have been developed which permit measurements of cytochemical activity without the need for synchronous cultures. Among these are autoradiography and tritiated thymidine treatment. Autoradiography consists of using, either the light or electron microscope to estimate the amount of a tracer by counting grains from autoradiographs of single cells. The most important use of autoradiography in cell cycle work has been to detect DNA synthesis by the incorporation of tritiated thymidine, a very specific label for DNA.

#### 4. Dynamics of Growth

Given the growth process of a cell, it eventually divides to become two cells. This takes place during the generation time or

division period. It is obvious, then, that if this continues, one no longer has one cell but a cell population. Thus, in the ideal case, if the original number of cells in a culture is  $N_0$ , then one generation later, when all these cells have divided, the number of cells is  $2N_0$ . At the end of the next generation period there would be 2 times  $2N_0$  and, so on. It follows, therefore, that the number,  $N$ , of cells at the end of  $g$  generation periods is given by

$$N = 2^g N_0 \quad (1-2)$$

If  $t_g$  is designated as the division period or generation time, then after time  $t$

$$g = t/t_g \quad (1-3)$$

By substituting equation (1-3) into (1-2) the following is obtained:

$$N = 2^{(t/t_g)} N_0 \quad (1-4)$$

This relationship is continuous, while a perfectly synchronous population would behave in a stepwise fashion. However, normal populations are asynchronous and the exponential expression above is approximately true for large numbers and becomes exact as the population increases without limit. It is apparent that if exponential growth could occur indefinitely, a bacterium with a generation time of 20 minutes would in one day give rise to a culture weighing some thousands of tons. There are, however, two rather obvious reasons why we are not up to our "butts" in bacteria. Not only would the increasing bacterial population rapidly run out of nutrient on which to feed, but their toxic waste products would

further inhibit their growth. Under normal circumstances such bacteria grow exponentially for a while until these inhibiting actions force the population into a so-called stationary phase during which there is no net increase in the number of the cells in the culture.

#### B. Cell Models

The cell is a complex entity which can be interpreted in a rather qualitative fashion by descriptive analysis. However, if more than a cursory and gross picture is to be drawn among the many functional units that make up this complex, a more quantitative and formalized system of analysis must be undertaken. This can be done by looking for an idealized mathematical model of the system.

Biological functions are, for the most part, complicated nonlinear processes, whereas a cursory descriptive analysis depends primarily upon linear logic. The results of more quantitative study via mathematical modeling could perhaps prompt the veteran descriptive analyst to make the counter-intuitive claim, "I wouldn't have suspected that!" Therefore the processes of cellular growth and replication are more readily comprehended when the relationships between the functional elements involved are correlated in a quantitative manner by establishing a mathematical model.

There are essentially three types of mathematical analyses or approaches used in developing mathematical models for cell biology research: 1) continuous analysis\* of differential equations for the

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\*A glossary of pertinent cell modeling terms will be found in Appendix C.

kinetics of metabolic systems, 2) finite mathematics of discrete states as those of automata theory, and 3) hybrid analysis which includes a combination of the continuous and the finite in a sort of logical control theory. These three approaches offer the most convenient and logical division for a review and comparison of previous modeling efforts. Several models with their various approaches have been published; a review of these studies will help to determine the balance between complexity and generality required and illustrate the need for the present investigation.

Table I-1 lists some of the more pertinent published work in the field of cell modeling and summarizes the approach and scope of the studies cited. The list, in chronological order, is separated into the three basic approaches.

### 1. Continuous Analysis

The earliest cell modeling work in the field, by Morowitz, et al. (1964) [42], is a logical extension of previous studies on simple enzyme systems. Spurred by an interest in passive stability of such a metabolic network, a model system consisting of a "biochemical vat" containing enzymes and small molecules was constructed. Morowitz considered all enzymatic reactions to be elementary ones, and excluded reactions that were coupled through spatial structures. Therefore, template construction and subsequent macromolecule production were not modeled. In this sense, Morowitz et al. have not modeled a cell and this was not their claim. However, their study does represent a higher degree of organization in comparison with the enzymatic studies previously performed. Their model was not

TABLE I-1  
Mathematical Models of Cells

<u>Investigator</u>	<u>Mathematical Approach</u>	<u>Quantitative</u>	<u>Type of Cells Modeled</u>	<u>Genetics Modeled? How?</u>	<u>Volume Modeled?</u>	<u>Made Data Comparison?</u>
Morowitz et al. [42] (1964)	Continuous Analysis	No	Bacterial	No	No	No
Yeisley and Pollard [43] (1964)	Continuous Analysis	Yes	Bacterial	Yes, DNA	No	No
Heinmets (a) [45] (1966)	Continuous Analysis	Yes	Any	Yes, Gene	No	No
Reiner [46] (1967)	Continuous Analysis	No	Bacterial	No	Yes	No
Weinberg [47] (1968)	Continuous Analysis	Yes	<u>E. coli</u>	Yes, Gene	Yes	Yes
Simon [48] (1973)	Continuous Analysis	Yes	<u>E. coli</u>	Yes, Gene	Yes	Yes

TABLE I-1 (continued)

## Mathematical Models of Cells

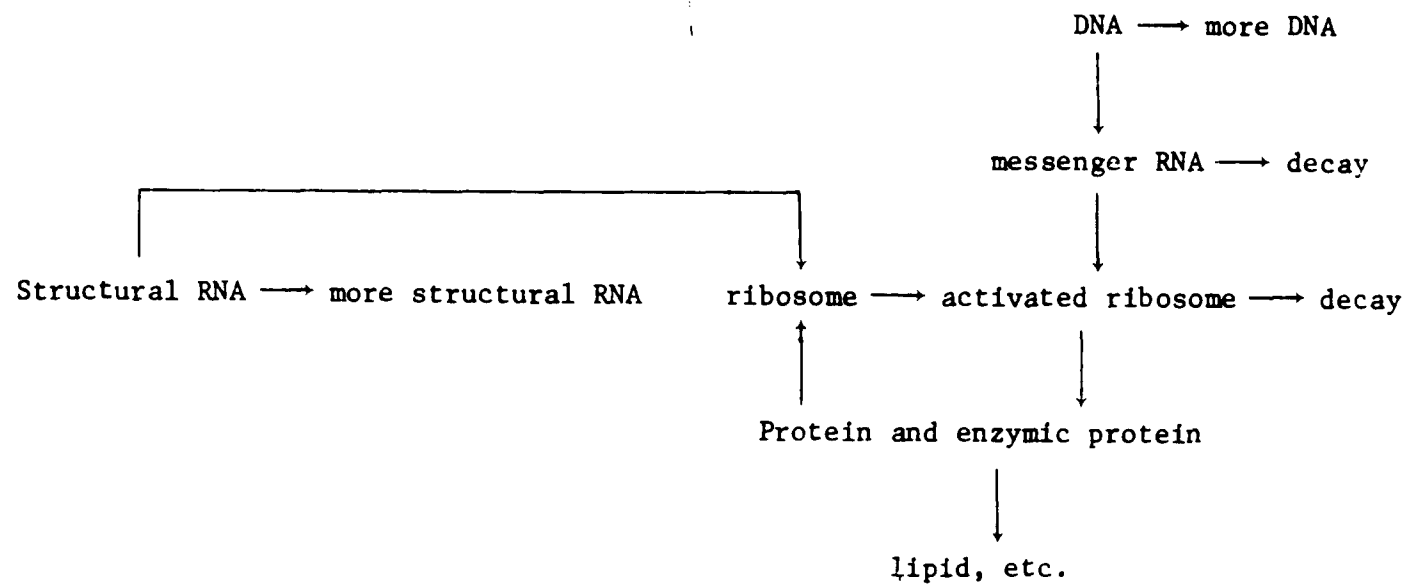
<u>Investigator</u>	<u>Mathematical Approach</u>	<u>Quantitative</u>	<u>Type of Cells Modeled</u>	<u>Genetics Modeled? How?</u>	<u>Volume Modeled?</u>	<u>Made Data Comparison?</u>
Stahl [50] (1967)	Automata Theory	No	Any	Yes, Gene	No	No
Heinmets (b) [45] (1966)	Logical Control	No	Any	Yes, Gene	No	No
Simon et al. [52] (1968)	Logical Control	No	Any	Yes, Gene	Yes	No
Sugita [53] (1968)	Logical Control	No	Any	Yes, Gene	No	No
Present Study	Logical Control	Yes	Any	Yes, DNA	Yes	Yes



quantitative since no actual chemical components or functional quantities were used in their analog computer simulation. Designed with stability studies in mind, the model suggests that a high degree of stability can be conferred upon a chemical vat system composed of only passive elements. The model is directed toward the study of bacterial systems but its generality does not prohibit its application to higher systems. The passive system thus established sets up a foundation upon which the active control of more sophisticated systems can be overlaid.

One of the more functional model systems developed is that of Yeisley and Pollard (1964) [43]. Not designed to model highly specific factors such as the coding of enzymes by DNA, it does however relate in a quantitative fashion the logistical elements within the bacterial cell. A schematic representation of the synthesis patterns modeled is shown in Figure I-2. DNA performed the two functions of replication and transcription while structural RNA formed the integral part of ribosomes.

Structural RNA was proposed to be autocatalytic, a definite misrepresentation of the mechanism discussed previously. Ribosomes, were formed by a combination of structural RNA and protein, and were subsequently activated by reacting with messenger RNA to form the protein-producing complex. Part of the protein produced combined with the ribosomes and part went to production of lipid. A necessary simplification of the analysis was achieved by Yeisley and Pollard by assuming other necessary reactants to be always present in excess. This is not always true but does appear representative for cells grown in relatively rich media. Another key feature of this



Model System of Yeisley and Pollard (1964) [43]

FIGURE I-2

model was the inclusion of both messenger RNA and activated ribosome decay. In bacterial systems, these degradations are extremely fast from a chemical point of view and, therefore, cannot be neglected.

Material balances were derived by Yeisley and Pollard in the form of nonlinear ordinary differential equations which tacitly assumed a constant volume for the cell system. This too, is clearly not an accurate picture. If no nonlinear or second-order reactions had been modeled, the constant and variable volume assumptions would respectively lead to similar results; but such is not the case. Realistic initial conditions for a bacterial system were chosen and by imposing the constraint that all parameter initial values be doubled at the end of one generation, a trial and error technique was instituted to obtain a set of rate constants consistent with both sets of conditions. It was determined experimentally that by fixing the decay rates of mRNA and activated ribosomes, the remaining constants were uniquely determined. The solutions to the cellular differential equations were in this sense unique.

The effects of various perturbations on the cell model were tested by these investigators. They included inhibiting first DNA, mRNA, activated ribosome, and protein synthesis in successive studies and followed each by making subsequent qualitative observations of the effect. No experimental data was used to support the model's results for normal growth.

Stability studies were also performed upon the cell model over a period of several generations or division cycles. In this regard, a stress was applied and the cell was allowed to "grow" until each of

its components had at least doubled for purposes of progeny survival after which cell division occurred. In a continuing fashion, if, after a number of generations the cell model restored itself to its original conditions, the "cell" was considered to be stable, or more properly asymptotically stable. If the progress of the "cell" was such that its components tended toward clearly divergent values, then the "cell" was termed unstable. In this fashion, Yeisley and Pollard demonstrated that their model of a bacterial cell appeared to be more stable to any one single stress than it was to simultaneous stresses. Fast messenger RNA decay improved stability and cyclical DNA synthesis (synthesis during only part of the cycle) likewise made the "cell" markedly more stable. The cell's freedom to change its growth cycle in response to stress was modeled by allowing an increase in the last phase of the cell cycle, the G2 period. Actual cells with different cycle times tend to have varying G1 periods, however, and relatively constant S + G2 [44]. The analog computer work performed by Yeisley and Pollard represents a benchmark study upon which the present investigation is based.

Heinmets, in his text entitled, Analysis of Normal and Abnormal Cell Growth (1966) [45], evaluated both a basic quantitative model, (a), and then an advanced qualitative cellular model, (b). In this section we will consider model (a).

In the text, a model system for cell growth was established and extensive kinetic analysis was carried out for the growth process. This model is a completely closed loop type as opposed to the earlier described chemical vat of Morowitz [42]. In this context, it included basic interactions among the various functional entities

such as genes, messengers, templates, enzymes, repressors, and activators. A single input into the system was provided from an external pool. Furthermore, the model contained several regulatory features related to the control of both enzyme synthesis and enzyme action. Heinmets performed his study via analog computer during the cell cycle with the exclusion of nuclear division. He investigated the growth of cellular entities as a function of a variety of simulated conditions.

Heinmets' mathematical approach is similar to that of Yeisley and Pollard. Reaction mechanisms in terms of material balances resulted in nonlinear first-order ordinary differential equations. In this case, however, fictitious initial conditions were picked and, as before, an overly simplified assumption of constant cell volume was made. The rate constants were again determined by trial and error. This time, however, the set of equations had no unique solution since there were more constants than boundary constraints. Heinmets argued that the number of functional systems establishable by this procedure is limited, but the underspecified nature of this system demands that only basic qualitative interpretations of these simulation results are reasonable. In general, validation of such studies requires the support of experimental data. However, the general idea of representing a network of interactions in a quantitative manner does help to change an "illusion of understanding" into real understanding.

As more and more information about the molecular biology aspects of cellular biosynthesis becomes evident, this information is integrated into newer, more complete model systems. For example, in

1967, Reiner [46] included the then current account of the steps for the synthesis of the relatively permanent components, the macromolecules, and applied it to deduce the kinetics of mass and volume growth in individual cells between divisions. To make the model more tractable, several assumptions were made, among which the saturation of certain mechanisms by the pools of precursors and the polymerases native to them, was key. In this study, moreover, volume was rightly considered to be variable, though for the sake of simplicity, spatial concentration gradients within the cell were neglected. This was tantamount to considering the cell as a lumped parameter system. Cell mass derivation yielded a linear growth expression while volume growth was nonlinear depending on the volume of cell water as well as on the volume of synthesized macromolecular mass. At the time of this study, Reiner had no experimental data that was adequate to test the validity of his model. Finally, although genetic information was inherent in the model assumption, it was, however, not evaluated as a separate entity.

Weinberg [47] in 1968, was the first biological researcher to utilize quantitatively experimental data to interpret his mathematical representation of a cell system, that of Escherichia coli. His simplified model of E. coli was composed of difference equations representing changes in the various chemical pools within a cell undergoing exponential growth. Since it is clear that in even the simplest of cells there are more than 3000 different kinds of molecules all interrelated in function, only a rather limited metabolic network consisting of several chemical pools was included in the model. The decision regarding which molecules belonged in

which pool was derived from a consideration of metabolic topology, functional relationships, and experimental data.

The program thus developed and solved digitally correctly predicted changes in the chemical constituents of an E. coli cell as verified by previously published experimental data. This study, therefore, represents the first successful implementation of the system theory concept of a biological model for computer simulation.

Simon's work just published [48] is the latest effort in the continuous analysis approach to the modeling of cellular systems. Like Weinberg, Simon formulated a model of the E. coli cell system based on the Helmstetter concept [49] which infers that a new round of DNA replication in a cell containing a certain number of chromosome ends is initiated when a threshold quantity of a division substance accumulates. In this model, enzyme synthesis occurred with competition for a common precursor and transcription was limited by the quantity of RNA polymerase. Media enrichment favored the activity of enzymes involved in cell division. The Simon model accurately predicted for E. coli a division rate inversely proportional to total gene activity, with a cell volume and number of chromosomes constant at low growth rates and proportional to the growth rate at high division rates. It also offered some explanation for the relative non-dependence of cell size and composition on temperature.

## 2. Automata Theory

The principle contribution to the field of biological research by cell modeling made by automata theory was that of Stahl [50] in

1967, utilizing a logic device called a Turing Machine [51]. The Turing Machine is a simple logical device which is basically a formalized means of writing symbols on a tape. Stahl modified this device for implementation by a digital computer using an addressed string search technique. The cell contents, then essentially consist of a set of lists of different cell constituents and structures. These included DNA composed of 46 genes, RNA, cell membranes and others. The model, a self-reproductive type, considered the following sequential events: (1) the discrete construction of requisite substrates, (2) the determination of the presence of all necessary strings for reproduction, (3) the shutting down of synthetic genes, (4) the separation of chromosomes, and finally, (5) cellular division.

There is much to recommend the "list-processing" approach to cell modeling, such as its ease in defining the cellular components in an orderly manner. Fairly smooth and gradual changes in composition can be achieved even if only based on an integral philosophy. Perhaps a study of the E. coli system in this manner would provide a worthwhile comparison for Weinberg's model [47]. Unfortunately, Stahl does not offer such a quantitative study supported by the necessary experimental data.

### 3. Logical Control Models

This type of model employs many of the continuous analysis concepts for enzymatic and metabolic processes within the cell while the genetic and mitotic processes are more suitably modeled in



finite terms. "Trigger" mechanisms, gene control, and replication initiation processes are most often handled in this manner.

In his text, Heinmets [45] made a qualitative analysis of an advanced cellular model system referenced previously as (b) in Table I-1. This model, although similar in many respects to his basic model of cell growth, included various regulatory and trigger mechanisms to model the division of genes and cellular division, as well as variations in the activity of genes due to repressors and activators. The most notable difference is that this latter model represents descriptive analysis and no attempt was made to obtain a solution, to simulate, or to further interpret.

Simon et al. (1968) [52] studied the function of a cell model in which DNA synthesis was assumed to start at a time when the concentration of necessary precursor reached a threshold value. The precursor was synthesized at a rate proportional to a mitotic enzyme and cell volume was proportional to total protein mass. To establish stable cellular behavior from cycle to cycle, the daughter cells after division were required to be identical with the parent cell at the beginning of the previous cycle. A digital computer study using fictitious rate constants and concentrations demonstrated that the cell model tended toward stable, periodic behavior. Designed to demonstrate cell function only, the model was neither quantitative nor data-validated.

Finally, Sugita (1968) [53] applied a method of interaction of continuous and finite systems to a model of cell division. The kinetics of the continuous system of the metabolites regulating mitosis and DNA replication were coupled with an automata modeling of

signals. A hybrid computer consisting of an analog computer with a relay circuit was used to study qualitatively this model system which consisted of a simplified version of Heinmets' rather complicated model for cell division and DNA duplication.

### C. Summary

A review of this first chapter indicates that we have prepared a foundation for the development of a model for the growth and replication processes which occur during the cell cycle of higher eukaryotes. We have presented a basic description of these biological processes from the standpoint of the kinetics of chemical syntheses involved as well as the functions of biological structures. This discussion led to a review of previous modeling efforts to analyze the growth and replication processes. As expected, the beginnings of these analyses were very elementary indeed, dealing only with biochemical pools or bacterial cells. More advanced models were developed, but with the notable exceptions of Weinberg [47] and Simon [48], there has been a lack of quantitative study in the field of cell modeling. Weinberg and Simon developed models of E. coli, whereas higher order cells have received only scant attention to date.

A better understanding of the functioning of eukaryotic cells is needed if their role as the basic unit of life in higher organisms is to be comprehended. As mentioned previously, very little data was available to verify earlier studies, but with this obstacle removed, higher order cell models with simulation capabilities are feasible.

Thus, the purpose of this research is to help fill the void that now exists in this area by developing a model which will attempt to provide a clearer and more quantitative understanding of these cell systems.

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## CHAPTER II

### MODEL DEVELOPMENT

The literature survey performed in Chapter I revealed that the only experimental data-validated models yet developed are those of the bacterium Escherichia coli. The realization that a complete model of an even simpler organism could easily involve as many as 2000 reactions relating 400 biochemicals, has surely had a deterrent effect upon earlier attempts to model the behavior of the more sophisticated cellular systems of animals, even with ready availability of the necessary data. Since a model often is an idealization of the actual system anyway, some understanding of basic interactions can be achieved even if the constituents involved represent only a small but representative portion of the total. Support for such a model is reaffirmed by feedback in the form of experimental data documenting the results of the model. Thus, a kinetic model of the cell cycle of higher eukaryotes will be developed in just this manner.

#### A. Derivation of Higher Eukaryote Cell Model

In order to develop a quantitative description of the eukaryotic cell during its cycle, some simplifications must be made. A simplified, lumped parameter representation of the cell provides the basis for the system description.

# 1. System Description

For purposes of this study, the wall of a single eukaryotic cell will define the physical boundary of the system in a thermodynamic sense. DNA, rRNA, mRNA, tRNA, activated ribosomes, aminoacyl tRNA, protein, pools, polymerases, enzymes, complexes, and water comprise the list of constituents in this model cell. By including these components it is possible to model the three fundamental processes of cell growth: replication, transcription, and translation. An outline of these components and processes is given in Table II-1. Written in reaction path format, a schematic of these processes, based on the discussion in Chapter I, is shown in Table II-2.

A careful listing of all limiting assumptions must be made in order to understand just what limitations the model is expected to have. The first two of these assumptions are important for simplifying the cell system.

Assumption 1. The initial amount of polymerase or enzyme necessary for the replication, transcription, and translation mechanisms is sufficient to saturate these systems independent of any new synthesis of polymerase or enzyme. In using the term "saturate", we imply in the usual kinetic sense that the reaction rates in these systems are independent of the amount of the reactant in excess because the quantity of that reactant remains essentially constant.

Assumption 2. The required precursors or pools necessary for synthesis of new macromolecules are present in excess.



TABLE II-1  
Eukaryotic Cell System Components and Their Functions

Component	Process(es) Involved
DNA	Replication, Transcription
rRNA	Transcription, Translation
mRNA	Transcription, Translation
tRNA	Transcription, Translation
Activated Ribosomes	Translation
Aminoacyl tRNA	Translation
Protein	Translation
Water	Many Processes
Pools	Many Processes
Polymerases	Many Processes
Enzymes	Many Processes

TABLE II-2

## Schematic of Principle Reaction Paths in Eukaryotic Cells

---

Original DNA + Pool	Polymerase →	Original DNA + New DNA	(2-1)
DNA + Pool	Polymerase →	rRNA + DNA	(2-2)
DNA + Pool	Polymerase →	mRNA + DNA	(2-3)
DNA + Pool	Polymerase →	tRNA + DNA	(2-4)
mRNA	→	decay products	(2-5)
mRNA + rRNA	→	activated ribosomes	(2-6)
tRNA + Pool	enzyme →	aminoacyl tRNA	(2-7)
aminoacyl tRNA + activated ribosome	→	complex	(2-8)
Complex	enzyme →	Protein + rRNA + mRNA + tRNA	(2-9)

---

Both Reiner [1] and Yeisley and Pollard [2] have indicated that these assumptions are readily justifiable under normal conditions. Cells in culture are generally grown in rich medium [3]. Therefore, by insuring an excess of certain reactants, simplified empirical mechanisms can be substituted for the more exact expressions. This procedure is typical in kinetic studies where an excess of one reactant leads to pseudo first-order reaction mechanisms.

All cellular constituents decay to some extent. However, the examination of relative stabilities in Chapter I indicated that the only cellular biochemical to suffer appreciable degradation was mRNA. Since the actual mechanism by which the breakdown occurs in eukaryotic cells is not fully understood, we shall assume simple exponential or first-order decay. Both Heinmets [4] and Yeisley and Pollard [2] concur with this simplified approach.

Assumption 3. Messenger RNA, the only cellular component that decays to any appreciable extent, does so in a first-order or exponential fashion.

The revised set of reaction mechanisms is depicted in Table II-3 where the  $k$ 's are rate constants with appropriate units.

Up to this point little has been said about cell volume. As was earlier cited, previous modeling efforts have often ignored this as a variable quantity. In this work it will be included. Volume growth of a cell essentially reflects two components: volume increase due to newly synthesized macromolecules and the increase of cell water content. Since eukaryotic cells are normally some 80% water, we introduce the following:

TABLE II-3

## Revised Set of Reaction Mechanisms

---

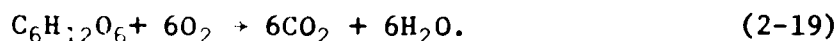
<u>Representation</u>	<u>Equations</u>
$D_0$ --- original DNA	$D_0 \xrightarrow{k_1} D \quad (2-10)$
$D$ --- total DNA	
$RR$ --- rRNA	$D \xrightarrow{k_2} RR + D \quad (2-11)$
$MR$ --- mRNA	$D \xrightarrow{k_3} MR + D \quad (2-12)$
$TR$ --- tRNA	$D \xrightarrow{k_4} TR + D \quad (2-13)$
$X$ --- decay products	$MR \xrightarrow{k_5} X \quad (2-14)$
$AR$ --- activated ribosome	$RR + MR \xrightarrow{k_6} AR \quad (2-15)$
$AT$ --- aminoacyl tRNA	$TR \xrightarrow{k_7} AT \quad (2-16)$
$ARAT$ --- complex for protein synthesis	$AR + AT \xrightarrow{k_8} ARAT \quad (2-17)$
$P$ --- protein	$ARAT \xrightarrow{k_9} P + RR + MR + TR \quad (2-18)$

---

Assumption 4. All volume change in the cell is considered to be due to new water.

Realizing that this water is used to balance the cell's content of newly synthesized macromolecules, any increase in cell macromolecular content (and consequently macromolecular volume) will be reflected by an increase in cell water. Reiner [1] indicates that the rate of volume increase due to the volume of newly synthesized macromolecules is normally an order of magnitude less than new water addition.

This necessary cell water may be provided in two ways, by metabolism and by mass flow. If we limit our discussion to cells growing under aerobic conditions or cells with a predominantly aerobic metabolism, the oxidation process is well known to produce water, e.g. a mole for every mole of oxygen consumed if, say, glucose is completely oxidized as demonstrated by the familiar chemical reaction



This would indicate that an actively respiring cell would produce, as it grows, the major part if not all of the water required to osmotically balance itself. Stephenson [5] has given a demonstration of this in an earlier study.

So, to an approximation, then,

Assumption 5. All new water is due to metabolism, not osmosis, and is proportional to oxygen consumption in cells growing aerobically.

What is a good measure of the oxygen consumption of an actively respiring cell? Experimental studies by Hershey and Bronfenbrenner

[6] and others as far back as 1938 have indicated that the rate of oxygen consumption in growing cells per quantity of cell nitrogen is roughly constant. Therefore,

Assumption 6. The rate of respiration is proportional to cell nitrogen content.

The most abundant nitrogen-containing substances in the cell are the protein materials. Each protein is constructed from amino acids which contain nitrogen in the form of  $\text{NH}_2$  groups. There is little error in saying, therefore, that

Assumption 7. The cell protein content is proportional to the cell nitrogen content.

Now, following our logic, we can see that if:

- (1) cell volume increase is due principally to new cell water,
- (2) the cell can furnish all its water by metabolism,
- (3) water formation is proportional to oxygen consumption,
- (4) oxygen consumption is in constant proportion to total nitrogen, thus
- (5) cell nitrogen is a reasonable measure of the total protein content,

hence we may say that the rate of cell volume increase is proportional to the total protein content of the cell. This can be written as

$$\text{Rate of volume increase} \propto \text{Protein} \quad (2-20)$$

and quantitatively

$$\text{Protein} \xrightarrow{k_{10}} \text{Volume.} \quad (2-21)$$

## 2. Material Balances

Normally both material and energy balances are required in the study of a reacting system. In this case, however, the heats of reaction are so low that heat exchange with surrounding medium is sufficient to eliminate temperature changes. Lehniger [7] indicated that cells essentially operate isothermally. Thus, if a cell is grown at constant temperature and there are essentially no thermal gradients within the cell itself, reaction rates and species compositions may be determined without the need of an accompanying energy balance.

Mass conservation for a given species can be generalized in molar terms for a time element  $\Delta t$  and a volume element  $\Delta V$  as

$$\begin{array}{ccccccc} \text{Moles of} & & \text{Moles of} & & \text{Moles of} & & \text{Accumulation} \\ \text{species } i & & \text{species } i & & \text{species } i & & \text{of reactant} \\ \text{fed to} & - & \text{leaving} & + & \text{formed in} & = & \text{in the} \\ \text{volume} & & \text{volume} & & \text{the volume} & & \text{volume} \\ \text{element} & & \text{element} & & \text{element} & & \text{element} \end{array} \quad (2-22)$$

The first two terms represent both the convective and diffusive transport of reactant  $i$  into and out of the reacting element in the time  $\Delta t$ . The third, or generation term, depends on the rate of reaction occurring within the volume element  $\Delta V$ . Little or no convective transport occurs at the cellular level, thus only diffusional processes account for distribution of cell material. Furthermore, in Appendix D we will demonstrate the following:

Assumption 8. Diffusion is rapid enough to insure that essentially no spatial concentration gradients exist.

Under these conditions, the volume element chosen for the balances becomes the volume  $V$  of the entire cell and the general balance simplifies to

$$\begin{array}{lcl} \text{Accumulation of species } i & = & \text{moles of species } i \\ \text{in cell} & & \text{formed in cell} \end{array} \quad (2-23)$$

The mass balance written for the  $i$ th component of a multicomponent system is then

$$r_i V \Delta t = \Delta n_i \quad (2-24)$$

where  $r_i$  is the rate of reaction for species  $i$ .

$\Delta n_i$  is the moles of species  $i$  formed in cell in the time  $\Delta t$ .

Dividing by  $\Delta t$ , taking the limit as  $\Delta t \rightarrow 0$ , and rearranging gives

$$r_i = \frac{1}{V} \frac{dn_i}{dt} \quad (2-25)$$

By the Law of Mass Action, the rate of each reaction will be proportional to the concentrations of each of the reactants.

Therefore, the resulting rates of formation of individual species are then determined as indicated in Table II-4. The one notable exception is the formation of DNA. By definition, DNA synthesis takes place only during the S period. Therefore, a "trigger mechanism" will be used to initiate and halt DNA synthesis. Mathematically, this takes the form of a step function such that



TABLE II-4

## Resultant Material Balance Equations

$$\frac{1}{V} \frac{dD}{dt} = k_1 \frac{D_0}{V} \cdot S(t) \quad (2-26)$$

$$\frac{1}{V} \frac{dRR}{dt} = k_2 \frac{D}{V} + k_6 \frac{RR}{V} \cdot \frac{MR}{V} + k_9 \frac{ARAT}{V} \quad (2-27)$$

$$\frac{1}{V} \frac{dMR}{dt} = k_3 \frac{D}{V} - k_5 \frac{MR}{V} - k_6 \frac{RR}{V} \cdot \frac{MR}{V} + k_9 \frac{ARAT}{V} \quad (2-28)$$

$$\frac{1}{V} \frac{dTR}{dt} = k_4 \frac{D}{V} - k_7 \frac{TR}{V} + k_9 \frac{ARAT}{V} \quad (2-29)$$

$$\frac{1}{V} \frac{dAR}{dt} = k_6 \frac{RR}{V} \cdot \frac{MR}{V} - k_8 \frac{AR}{V} \cdot \frac{AT}{V} \quad (2-30)$$

$$\frac{1}{V} \frac{dAT}{dt} = k_7 \frac{TR}{V} - k_8 \frac{AR}{V} \cdot \frac{AT}{V} \quad (2-31)$$

$$\frac{1}{V} \frac{dARAT}{dt} = k_8 \frac{AR}{V} \cdot \frac{AT}{V} - k_9 \frac{ARAT}{V} \quad (2-32)$$

$$\frac{1}{V} \frac{dP}{dt} = k_9 \frac{ARAT}{V} \quad (2-33)$$

$$\frac{dV}{dt} = k_{10} P \quad (2-34)$$

$$S(t) = 0 \quad 0 \leq t \leq t_S \quad (2-35)$$

$$S(t) = 1 \quad t_S \leq t \leq t_{G2} \quad (2-36)$$

$$S(t) = 0 \quad t_{G2} < t \leq t_g \quad (2-37)$$

where  $S(t)$  is the unit step function from  $t_S$  to  $t_{G2}$ ,  
 $t_S$  is the time at which the S period starts,  
 $t_{G2}$  is the time at which the G2 period starts,  
 $t_g$  is the total cell cycle time.

This technique is similar to the logic control schemes employed by Sugita [8] in his hybrid model system.

### 3. Initial and Final Conditions

As described previously in Chapter I, syntheses occur within the cell which double the quantity of each cell component. This insures that two equal daughter cells, identical to the original cell at the beginning of the cell cycle, will be formed. Actually, there is some statistical variation from this precise requirement; but on the average an equitable division of cellular material will take place.

Thus, for any given cell cycle, the initial conditions for our kinetic scheme are simply the initial numbers of moles and initial volume, i.e.,

$$n_i(0) = n_{i0} \quad (2-38)$$

and  $V(0) = V_0 \quad (2-39)$

where  $n_{i0}$  is the initial number of moles of species  $i$  at time 0,  
 $V_0$  is the initial volume of the cell.

Similarly, in order to satisfy the doubling criteria we must require that

$$n_i(t_g) = 2n_{i0} \quad (2-40)$$

$$V(t_g) = 2V_0. \quad (2-41)$$

### B. Development of Dimensionless Cell Equations

In many problems a change of variable greatly simplifies the form of the equations involved and also makes the equations easier to solve. In most cases the new variables will be both dimensionless and normalized. More important in this study is another advantage of changes of variable, namely, that the solution to the problem can be made more general. In this fashion, the cell equations will be transformed into general form, requiring only the value of  $G_1$ ,  $S$ , and  $G_2$  times as necessary input data.

#### 1. Material Balances

Here, the normalization procedure requires that the mass of each species be divided by its initial amount. Thus

$$\eta_i = \frac{n_i}{n_{i0}} \quad (2-42)$$

where  $\eta_i$  is the normalized number of moles of species  $i$ .

In a similar fashion, the cell volume is normalized by its initial value,  $V_0$ :

$$v = \frac{V}{V_0} \quad (2-43)$$

where  $v$  is the normalized cell volume.

Time is normalized by means of  $t_g$ , the generation time:

$$\theta = \frac{t}{t_g} \quad (2-44)$$

where  $\theta$  is the normalized cell generation time.

After making these substitutions, the material balance for cell protein can be written

$$\frac{P_0}{V_0 t_g} \frac{1}{\left[\frac{V}{V_0}\right]} \frac{d\left[\frac{P}{P_0}\right]}{d\left[\frac{t}{t_g}\right]} = k_9 \frac{ARAT_0 \left[\frac{ARAT}{ARAT_0}\right]}{V_0 \left[\frac{V}{V_0}\right]} \quad (2-45)$$

or

$$\frac{1}{v} \frac{dp}{d\theta} = \left[ \frac{k_9 \cdot ARAT_0 \cdot t_g}{P_0} \right] \cdot \frac{arat}{v} \quad (2-46)$$

By combining the constants into a single term, Equation (2-46) can be written as:

$$\frac{1}{v} \frac{dp}{d\theta} = k'_9 \cdot \frac{arat}{v} \quad (2-47)$$

which is of the same form as Eq. (2-33) with normalized variables substituted for the original ones and a new rate constant  $k'_9$ , which

incorporates the introduced constants, in place of  $k_g$ . A similar analysis for the remaining differential equations provides a new set of normalized and dimensionless equations outlined in Table II-5 (lower case letters refer to normalized variables). Thus, in order to compare the results of the model with experimental data, only the cell cycle time information plus the initial quantity of the particular component to be studied need be supplied.

## 2. Initial and Final Conditions

By normalizing the initial and final values of the component quantities and the cell volume, the following additional equations can be written:

$$\eta_1(0) = 1 \quad (2-57)$$

$$V(0) = 1 \quad (2-58)$$

$$\eta_1(1) = 2 \quad (2-59)$$

$$V(1) = 2 \quad (2-60)$$

In addition, the function  $S(t)$  becomes

$$S(\theta) = 0 \quad 0 \leq \theta < \theta_S \quad (2-61)$$

$$S(\theta) = 1 \quad \theta_S \leq \theta \leq \theta_{G2} \quad (2-62)$$

$$S(\theta) = 0 \quad \theta_{G2} < \theta \leq 1 \quad (2-63)$$

where the  $\theta$ 's are defined in a manner analogous to the previous  $t$ 's with the exception of the normalization factor,  $t_g$ .

TABLE II-5

## Normalized Material Balance Equations

$$\frac{1}{v} \frac{d(d)}{d\theta} = k'_1 \frac{d}{v} \cdot S(\theta) \quad (2-48)$$

$$\frac{1}{v} \frac{d(rr)}{d\theta} = k'_2 \frac{d}{v} - k'_6 \frac{rr}{v} \cdot \frac{mr}{v} + k'_9 \frac{arat}{v} \quad (2-49)$$

$$\frac{1}{v} \frac{d(mr)}{d\theta} = k'_3 \frac{d}{v} - k'_5 \frac{mr}{v} - k'_6 \frac{rr}{v} \cdot \frac{mr}{v} + k'_9 \frac{arat}{v} \quad (2-50)$$

$$\frac{1}{v} \frac{d(tr)}{d\theta} = k'_4 \frac{d}{v} - k'_7 \frac{tr}{v} + k'_9 \frac{arat}{v} \quad (2-51)$$

$$\frac{1}{v} \frac{d(ar)}{d\theta} = k'_6 \frac{rr}{v} \cdot \frac{mr}{v} - k'_8 \frac{ar}{v} \cdot \frac{at}{v} \quad (2-52)$$

$$\frac{1}{v} \frac{d(at)}{d\theta} = k'_7 \frac{tr}{v} - k'_8 \frac{ar}{v} \cdot \frac{at}{v} \quad (2-53)$$

$$\frac{1}{v} \frac{d(arat)}{d\theta} = k'_8 \frac{ar}{v} \cdot \frac{at}{v} - k'_9 \frac{arat}{v} \quad (2-54)$$

$$\frac{1}{v} \frac{d(p)}{d\theta} = k'_9 \frac{arat}{v} \quad (2-55)$$

$$\frac{dv}{d\theta} = k'_{10} p \quad (2-56)$$

### 3. Messenger RNA Decay

One additional piece of information is necessary. There is a broad difference in the decay rates of various different mRNA species. This model is limited to but one mRNA species. An examination of the literature [9] reveals that while there is a large variation, the range of values of decay times seems to bracket the average generation time for the cells in question.

Assumption 9. The decay time of messenger RNA, based on available data, is assumed to be of the same order as the generation time.

If messenger RNA decay is considered to be another mechanism in the metabolic processes of the cell, then, an estimate of an average decay time for mRNA based on generation time data, a measure of metabolic rate, is not unreasonable. Since, in normalized terms, generation time is identically 1, it follows that, based on first-order decay, the rate constant (reciprocal of the time constant) will also be identically 1. Thus;

$$k'_5 = 1. \quad (2-64)$$

### C. Establishment of an Operational Cell Growth System

In his text [4], Heinmets speaks of establishing a functional system. The principal characteristic of a functional system is the requirement that during the generation time all cell components double their initial value. This presupposes that rate constants representing synthesis of various entities should also be in proper relation to each other. The highly nonlinear characteristics of the cell system model make this proper relationship difficult to

achieve. To establish a functional system, then, the values of the reaction rate constants must be determined which will best satisfy the requirements that all cellular components double in the time of a cell cycle. Due to the nonlinear nature of the system differential equations, an analytical solution was not easily available and therefore was not sought in this study.

### 1. Numerical Schemes

It becomes clear, that we must resort to an implicit method for evaluating the rate constants. Thus, a "trial and error" scheme in the form of a search technique will be employed. This, coupled with an integration algorithm for solution of the differential equations, will provide the tools by which a viable solution to the system model can be achieved.

In order to obtain such a solution, the procedure that follows was developed and used.

- (i) We first initialized the problem with the cell initial conditions as given by Equations (2-57) and (2-58).
- (ii) We, then, assumed values for the 9 rate constants,  $k'_1 - k'_4$ ,  $k'_6 - k'_{10}$ , in a "trial and error" fashion chosen by a search technique.
- (iii) The coupled system of Equations (2-48) through (2-56) and (2-61) through (2-63) were integrated and solved.
- (iv) Using the final values obtained for the components and volume, we ascertained whether they were indeed double the initial values. If so, then the  $k$ 's chosen were correct; if not, then we repeated steps (i) through (iv).



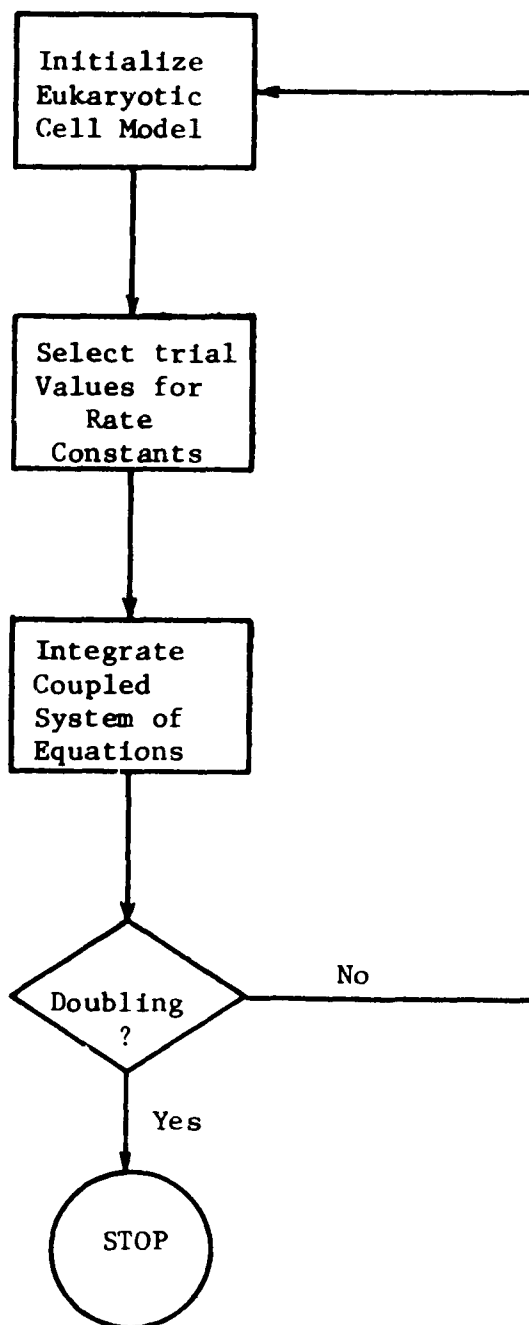
A logic flow diagram for the above procedure is given in Figure II-1.

There are two major concerns normally associated with a procedure such as this one. In the first case, it is important that the numerical integration scheme selected be flexible enough to handle the nonlinearities of the equation set, yet sufficiently rapid converging so as not to make the use of a search technique prohibitive. The other major difficulty is that the search technique employed must be very efficient because of the long computation time associated with repeated evaluations of the cost function representing the numerical solution of differential equations. Numerical schemes were selected with these two concerns in mind.

a) Integration Scheme

A fifth-order modified Runge-Kutta (MRK) integration algorithm devised by A. S. Chai [10] was employed. Using a linear combination of the quantities in the last integration step to replace the second derivative evaluation in the fourth-order unmodified Runge-Kutta formula (URK), this formula is used inclusively in a fifth-order scheme. A truncation-error estimate is obtained as the difference between the fourth and fifth-order formulas. In addition, the fifth-order MRK formula requires two less derivative evaluations at each integration step than the fifth-order URK formula due to the linear combination technique employed.

Most often, computation time is primarily used in derivative evaluation of a complex system of differential equations. Predictor-corrector methods need at least two derivative evaluations. The Chai



Logic Flow Diagram for Establishing a Functional Cell Model

FIGURE II-1

fifth-order MRK method needs four derivative evaluations, and hence, the computing time by this technique may be twice that used by the predictor-corrector scheme. The MRK method, however, has the redeeming advantages of a simple initiation procedure and the relative ease by which the integration step-size can be changed. In this problem with its nonlinear interaction, a variable step-size scheme is necessary to insure the combined effect of speed and accuracy which offers adequate compensation for the added derivative evaluations. For example, this scheme was consistently found to be more satisfactory than the corresponding fixed or variable step fourth-order unmodified Runge-Kutta when programmed in FORTRAN and run on either the XDS Sigma 5 or IBM 360/65 digital computers.

#### b) Search Technique

A multidimensional search, such as the one required here, has two drawbacks; first, there is no true measure of effectiveness as there is for unidimensional techniques; and secondly, the region of uncertainty must necessarily be but a tiny fraction of the original experimental region. In simpler terms there is no guarantee of discovering the "best" answers for the system in question. In a system which is known to have a unique solution, this problem is sometimes of little concern. But the equation set in question, even though it may appear completely specified with nine parameters and nine conditions to be met, is nonlinear and mathematically might therefore have multiple solutions. If a set of rate constants is found that satisfies the specified final conditions, it may not be the only set. This does not mean to imply that the biological system

has multiple states or solutions, but it simply requires that we check the rate constant values determined by the search technique to insure that they are indeed biologically realistic. We shall consider this particular problem in more detail later.

Several multivariable search techniques were tested for use in this study. These included a Pattern Search [11], Powell's Technique [12], and a Modified Powell Technique devised by Professor A. J. McPhate of LSU, as outlined by Özelsel [13]. Pattern Search and Powell's method both require a rather detailed knowledge of the system to be optimized or searched. It is also necessary to specify several "tuning parameters" from which these systems derive their search strategy. Modified Powell is so-named because of an important modification in the minimum evaluation; here, the normal quadratic fit to the response surface has been replaced by a cubic interpolating algorithm to approximate the relative minimum of the multivariable function.

Again, by actual test, the Modified Powell Technique of McPhate proved to be not only convergent but more quickly convergent than any of the others investigated. Requiring little more input information than starting values, the Modified Powell method provided much computing facility for resolving the multivariable search in question.

## 2. Demonstration of the Existence of a Single, Physically Meaningful Solution

We shall return now to reconsider in more detail the problems involved in relating the results of a limited search to those of a

unique solution. In order then to lend validity to the outcome of the multiparameter search performed here, certain precautions were taken:

- (i) Rate constants chosen by the search scheme were constrained to positive or zero values to preserve the mechanisms of the one-way transfer of information layed down by the "Central Dogma" as described in Chapter I.
- (ii) The region of search was designed to encompass realistic, or physically meaningful values of the parameters in question.

We implemented the first precaution by performing a "constrained" optimization of search, through use of a penalty function. In this method, a large penalty added to the calculated cost function discourages the computer's selection of physically unrealistic parameter values. If the choice of parameter values strays close to the established boundary, it too results in a proportional penalty. The words, "large", "close", and "proportional", are related to the normal value of the cost function which here has been calculated to be the sum of the squares of the differences between the final mass values determined for each specie and the required final values.

The second precaution taken is not so clear cut. We must direct the search toward a relatively small region of space. If some physically realistic estimates of the problem parameters are contained in this region, the search can be forced to bracket them. With this in mind, the following argument is proffered.

If experimental values of molar quantities for any component are available over a time period  $\Delta t$ , then the average rate of formation for that species can be written as,

$$\frac{\Delta n_i}{\Delta t} = \begin{array}{l} \text{average rate of formation} \\ \text{of component } i. \end{array} \quad (2-65)$$

In normalized form it becomes

$$\frac{\frac{\Delta n_i}{n_{i0}}}{\frac{t}{t_g}} = \text{average rate normalized.} \quad (2-66)$$

Furthermore, if these average normalized rates are evaluated over the cell cycle time, i.e.

$$\Delta t = t_g, \quad (2-67)$$

then the doubling requirement states that

$$\Delta n_i = n_{i0}. \quad (2-68)$$

When substituted into Equation (2-66), this yields,

$$\frac{\frac{n_{i0}}{n_{i0}}}{\frac{t}{t_g}} = 1 = \begin{array}{l} \text{average net rate} \\ \text{normalized.} \end{array} \quad (2-69)$$

The concept of exact doubling gives a value of 1 for all rates except that of DNA which is synthesized only during the S period. In this case

$$\Delta t = S \quad (2-70)$$

and so (2-69) becomes

$$\frac{\frac{n_{i0}}{n_{10}}}{\frac{S}{t_g}} = \frac{t_g}{S} = \begin{array}{l} \text{average net rate} \\ \text{of DNA synthesis} \\ \text{normalized} \end{array} \quad (2-71)$$

These values represent reasonable estimates for the net synthesis rates. A knowledge of these quantities and, say, the initial values of the normalized component amounts reduces the equation set (2-48) through (2-56) to a nine by nine set of linear algebraic equations in the k's, easily solved (explicitly in this case) for a reasonable set of starting values for the rate constants. In order to bracket these values, starting values were made at one-tenth and ten times these average values. The results of searches initiated from these three starting points, within the accuracy of the data utilized, indicated the existence of a single solution to the model system equations located within the region of biological feasibility as set up by these starting values. Comparative results are given in Table II-6.

TABLE II-6

Comparison of the Results of a Bounded Search Over a Range of Rate Constants

Optimum Values of Rate Constants									
Starting Values Based On	$k_1$	$k_2$	$k_3$	$k_4$	$k_6$	$k_7$	$k_8$	$k_9$	$k_{10}$
Average values for net rates	2.7137	2.0705	3.0262	2.0705	2.2169	2.1650	1.4398	0.6906	0.6916
0.1 x average values	2.7146	2.0696	3.0250	2.0697	2.2165	2.1648	1.4394	0.6911	0.6915
10 x average values	2.7142	2.0702	3.0257	2.0703	2.2169	2.1653	1.4397	0.6909	0.6916

For this example:  $G_1/t_g = 0.3684$

$S/t_g = 0.3684$

$G_2/t_g = 0.2632$

$t_g/t_g = 1.0000$



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## CHAPTER III

### RESULTS

A mathematical model describing dynamic isothermal growth of the higher eukaryotic cell during its cell cycle was developed in Chapter II. A numerical technique for establishing a functional cell system based on doubling criteria was introduced and quantitatively substantiated. In this chapter, a simulation of the growth and replication processes will be performed in an effort to shed some light on the interactions involved and possible causal relationships.

#### A. Normal Growth Studies

The simulation of the normal growth of the eukaryotic cell via a digital-computer-implemented numerical scheme has been achieved by a method similar to that of Yeisley and Pollard [1]. After we determined the rate constant values which satisfied the doubling criteria, these parameters were used to rerun the integration of the cell material balance equations over a normalized time period comparable to the generation time. Realizing that only the doubling criteria and the literature-supplied experimental data of G<sub>1</sub>, S, and G<sub>2</sub> times were used in the scheme for rate constant determination, the simulation run provides the dynamic response of each of the cellular constituents as well as volume. Comprehensive experimental data from any one single cell type is not currently available for comparison. However, from models of several cell types (different

G1, S, and G2 periods), a comparison with experimental results was obtained for DNA, rRNA, mRNA, tRNA, protein and cell volume.

### 1. DNA

The pattern of DNA synthesis in eukaryotic cells is the most thoroughly explored region in cell cycle studies. The fact that DNA has the singular importance of being the genetic material of cells justifies much of the effort spent on it. Because DNA synthesis occurs during a part of the cell cycle (S period), its study is somewhat simplified.

The DNA cycle of mouse fibroblasts was selected because it is a typical and well worked out example of the pattern in mammalian cells. Zetterberg and Killander have performed several studies [2, 3] on the interphase growth of these cells in vitro. In their work, cells taken from monolayer cultures in log phase growth were deposited on slides which were repeatedly photographed in a microscope for age determination. Randomly selected cells from these cultures were then analyzed individually for DNA content as determined by microspectrophotometric examination at  $5460 \text{ \AA}$  after Feulgen staining (a DNA-specific staining technique). The generation time in the second study was 19 hours and the mitotic time could be neglected. The lengths of the G1, S, and G2 periods were estimated from the DNA synthesis curve to be 9.4, 6.1, and 3.5 hours, respectively. This second study represented an improvement over the first, for the variation in DNA content among cells in the same physiological stage of the cell cycle was corrected. This statistical variation would ordinarily cause the DNA content measurements to fall outside its

theoretical limits. This sample distribution data, together with the eukaryotic cell model (ECM) results are shown in Figure III-1.

The data points are those of Zetterberg and Killander [3], which were abstracted from their DNA histogram based on a study of 594 cells. During the S period DNA was synthesized at a constant rate as evidenced by both model and data. The model description of DNA synthesis requires such a rate and the derived synthesis curve for mouse fibroblasts supports this thesis. An assumption of the ECM is that DNA synthesis does not occur outside of the S period, and, hence, it cannot predict the small amount of synthesis in the G1 and G2 periods exhibited by the data in the previous figure. Until now, the only DNA that was considered has been that in the nucleus. Recently, Holt and Burney [4] have established that a small fraction of DNA is contained in the cytoplasm, and that the pattern of replication of this DNA fraction is continuous. This could account for the deviation of the experimental data from the discontinuous form predicted by the ECM simulation. In this work the ECM does not consider cytoplasmic DNA synthesis.

## 2. RNA Fractions

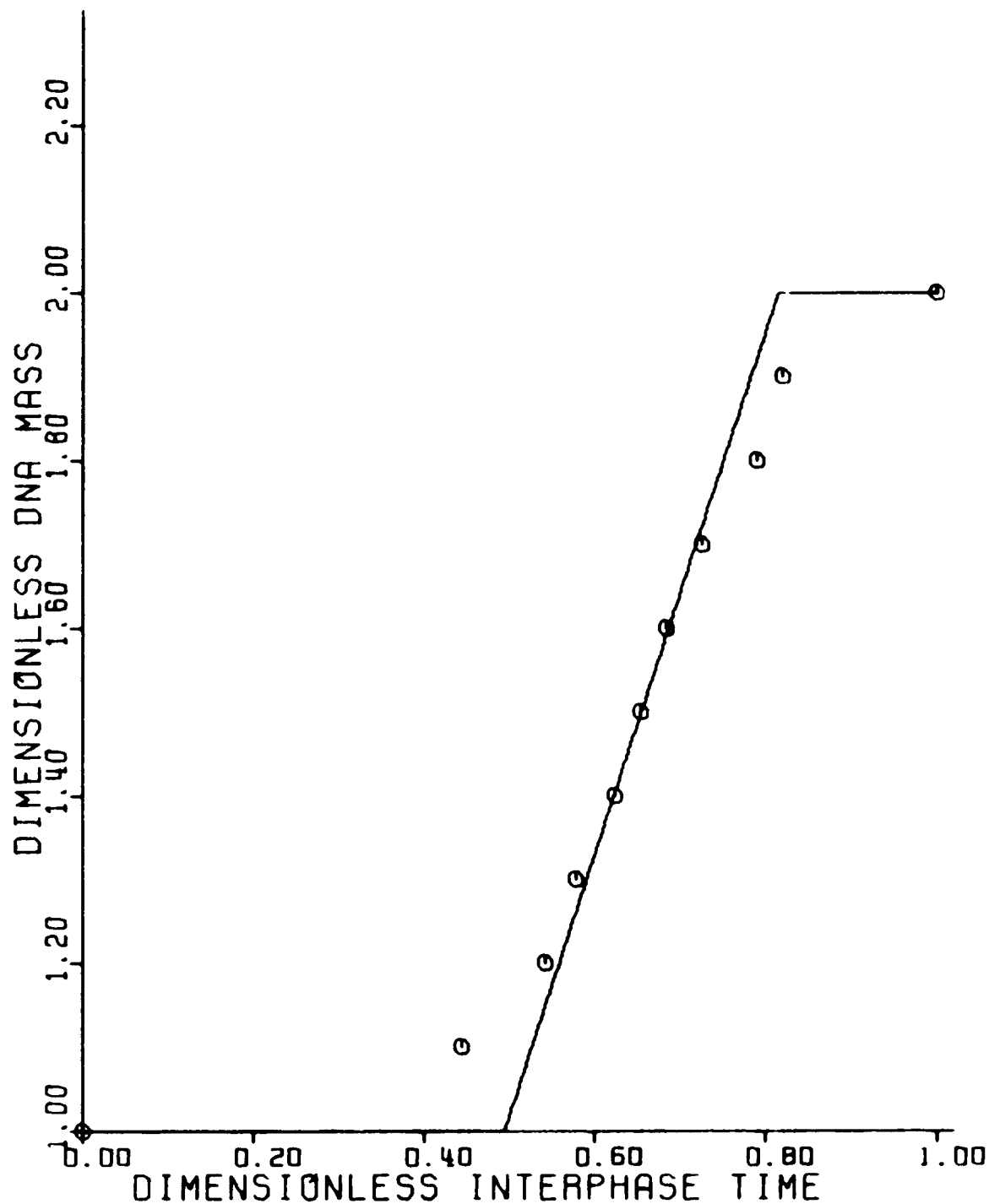
Ribonucleic acid synthesis presents a new problem. Unlike DNA, cellular RNA consists of many different fractions including the principal ones: rRNA, mRNA, and tRNA. This compounds the difficulties of interpreting the detailed studies of the rates of RNA synthesis during the cycle. Again, Zetterberg and Killander [2, 3] provide information on the overall pattern of total RNA synthesis, but only

## FIGURE III-1

DNA IN MOUSE FIBROBLASTS DURING NORMAL GROWTH

## LEGEND

- EUKARYOTIC CELL MODEL UNDER NORMAL CONDITIONS
- DATA FROM FEULGEN STAINS. ZETTERBERG AND KILLANDER (1965)



Pfeiffer [5] has made the necessary separation of the RNA into the appropriate fractions.

Utilizing a synchronous population of HeLa S3 cells (a strain of human cancer cells), Pfeiffer determined the rates of synthesis of RNA species by measuring the incorporation of  $C^{14}$ -uridine into the appropriate cellular fraction. Whole cell RNA, nuclear fraction, transfer RNA and messenger RNA synthesis patterns in terms of rates versus cell age were developed. From the labeling techniques that he employed, Pfeiffer was able to ascertain that the incorporation of precursor into the nuclear fraction most closely represents the synthesis of ribosomal RNA.

The rate data of Pfeiffer was graphically integrated to provide quantity versus time profiles for comparison with the ECM. Sample data points from each of these integrations are shown with the results of the ECM for the fractions rRNA, mRNA, and tRNA in Figures III-2, III-3, and III-4 in the form of a normalized mass increase versus time. Experimentally determined G1, S, and G2 times of 7, 7, and 5 hours respectively [6], were utilized as input data to the ECM.

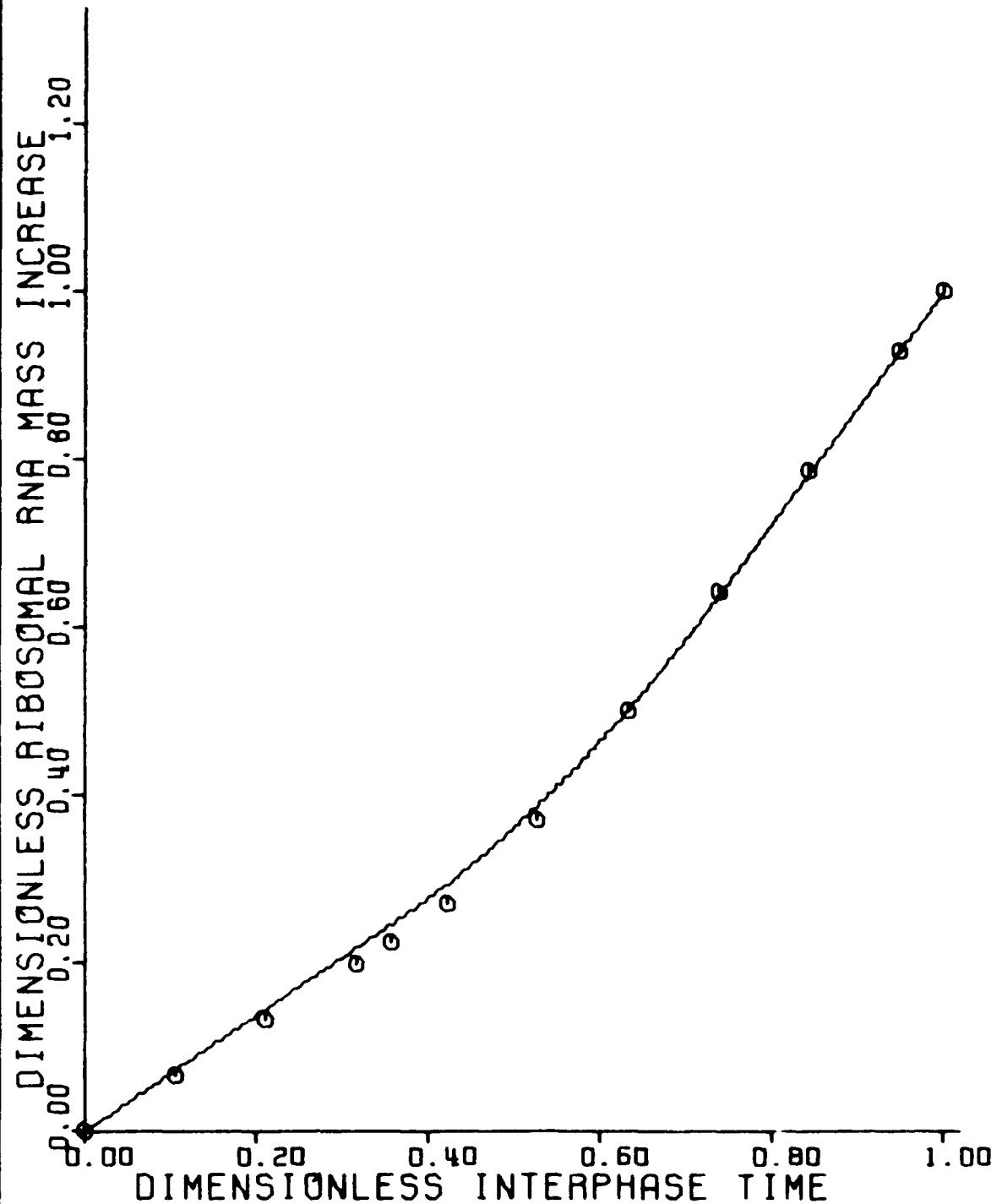
The synthesis of all three RNA species occurs throughout interphase in both data and the ECM, but undergoes an increase during the S period which is dependent upon the duplication of DNA. In fact, closer analysis reveals that both data and model show an approximately doubled rate by the end of S. This gene-dosage effect, anticipated because of the mechanisms of the transcription process, is verified by close comparison between Pfeiffer's integrated data and the ECM simulation results.

## FIGURE III-2

RIBOSOMAL RNA IN HELA S3 CELLS DURING NORMAL GROWTH

## LEGEND

- EUKARYOTIC CELL MODEL UNDER NORMAL CONDITIONS  
O DATA FROM C14-URIDINE INCORPORATION. PFEIFFER (1968)

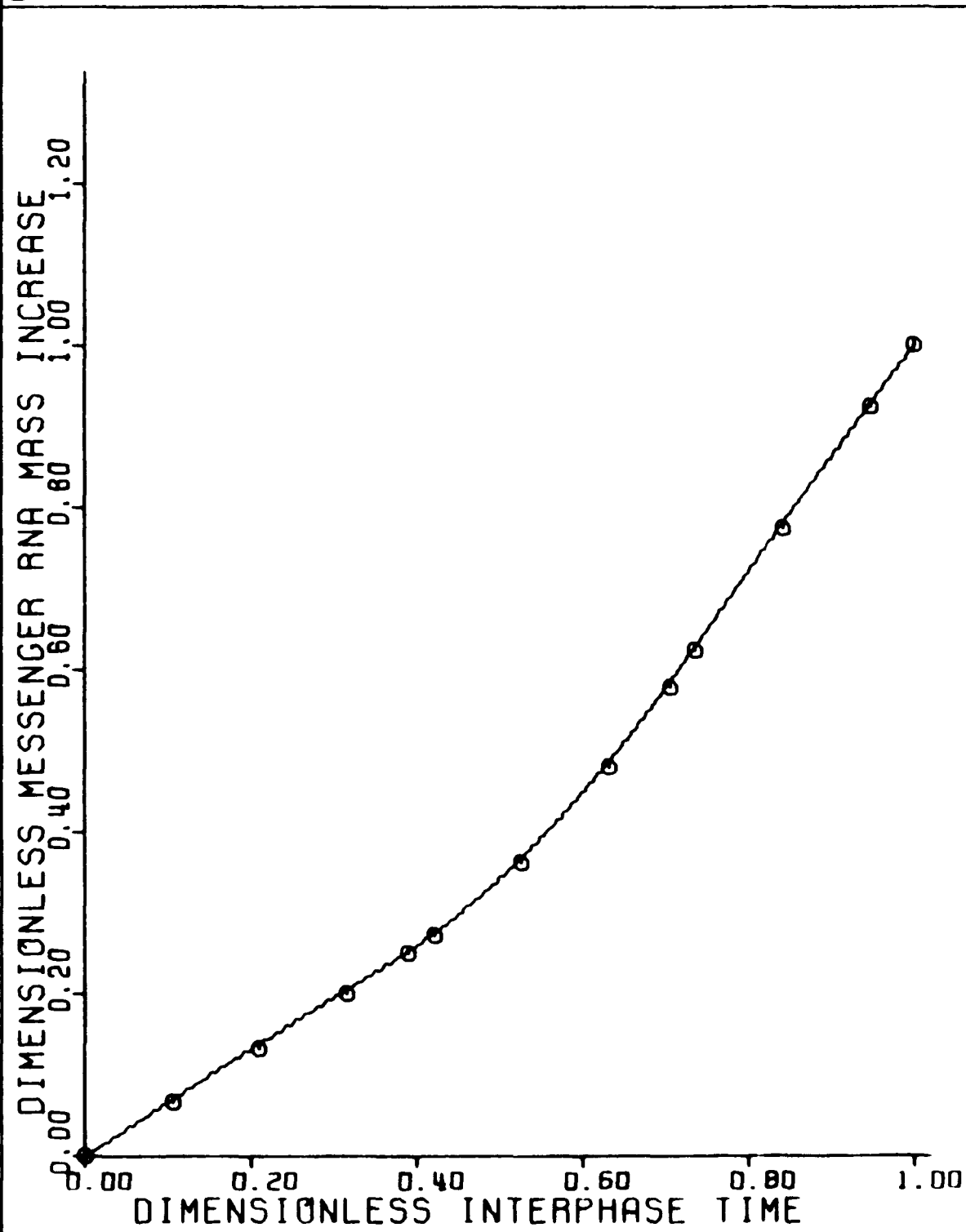


## FIGURE III-3

MESSENGER RNA IN HELA S3 CELLS DURING NORMAL GROWTH

## LEGEND

- EUKARYOTIC CELL MODEL UNDER NORMAL CONDITIONS  
O DATA FROM C14-URIDINE INCORPORATION, PFEIFFER (1968)



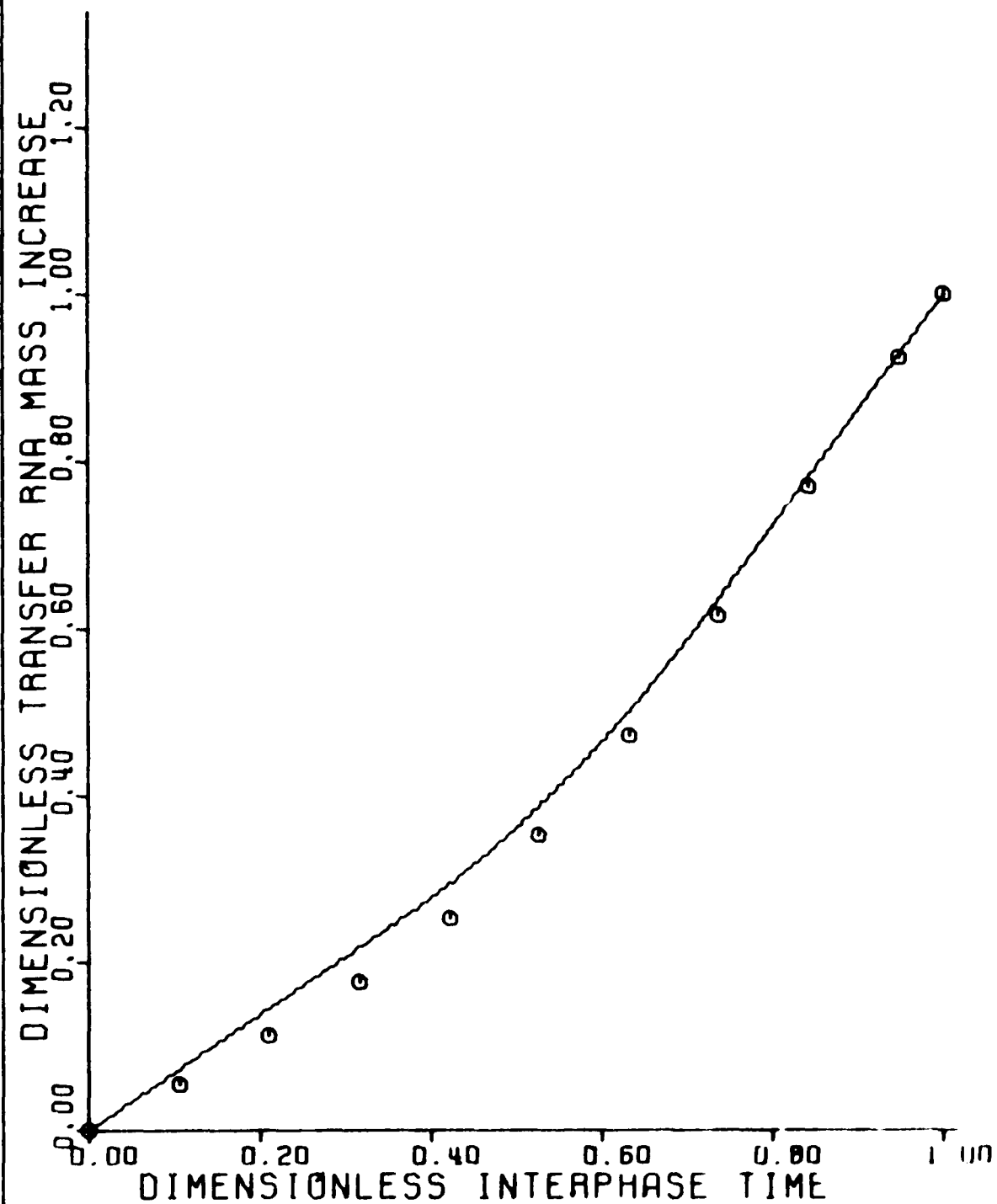


## FIGURE III-4

TRANSFER RNA IN HELA S3 CELLS DURING NORMAL GROWTH

## LEGEND

- EUKARYOTIC CELL MODEL UNDER NORMAL CONDITIONS  
O DATA FROM C14-URIDINE INCORPORATION, PFEIFFER (1968)



### 3. Protein

Protein material is by far the largest macromolecular component of most growing eukaryotic cells, specifically animal cells. Some of the best information about the overall pattern of synthesis in higher eukaryotic cells comes, again, from Zetterberg and Killander [7]. The rate of protein synthesis was investigated with quantitative autoradiographic techniques after pulse incubations of interphase cells with labeled amino acids.

Mouse fibroblasts were cultivated directly on slides and a pertinent field was photographed by time-lapse cinematography for purposes of age determination. Cells were fixed (growth stopped) at various ages and autoradiographic techniques were used in determining uptake of the radioactive labeled amino acids. In three separate experiments Zetterberg and coworkers added  $C^{14}$ -leucine,  $H^3$ -leucine, or  $S^{35}$ -methionine to the growth medium before fixing.

The rate of protein synthesis during interphase, as represented by the total number of grains (on film) per individual cell was graphically integrated for this study to provide mass versus time profiles for comparison in a normalized fashion with the ECM. Sample data points from these integrations for each of the labeled precursors are shown with the results of the ECM in Figure III-5 in the form of normalized mass versus time. Experimentally determined G1, S, and G2 times of 8, 6, and 5 hours respectively [2], were utilized as input data to the ECM.

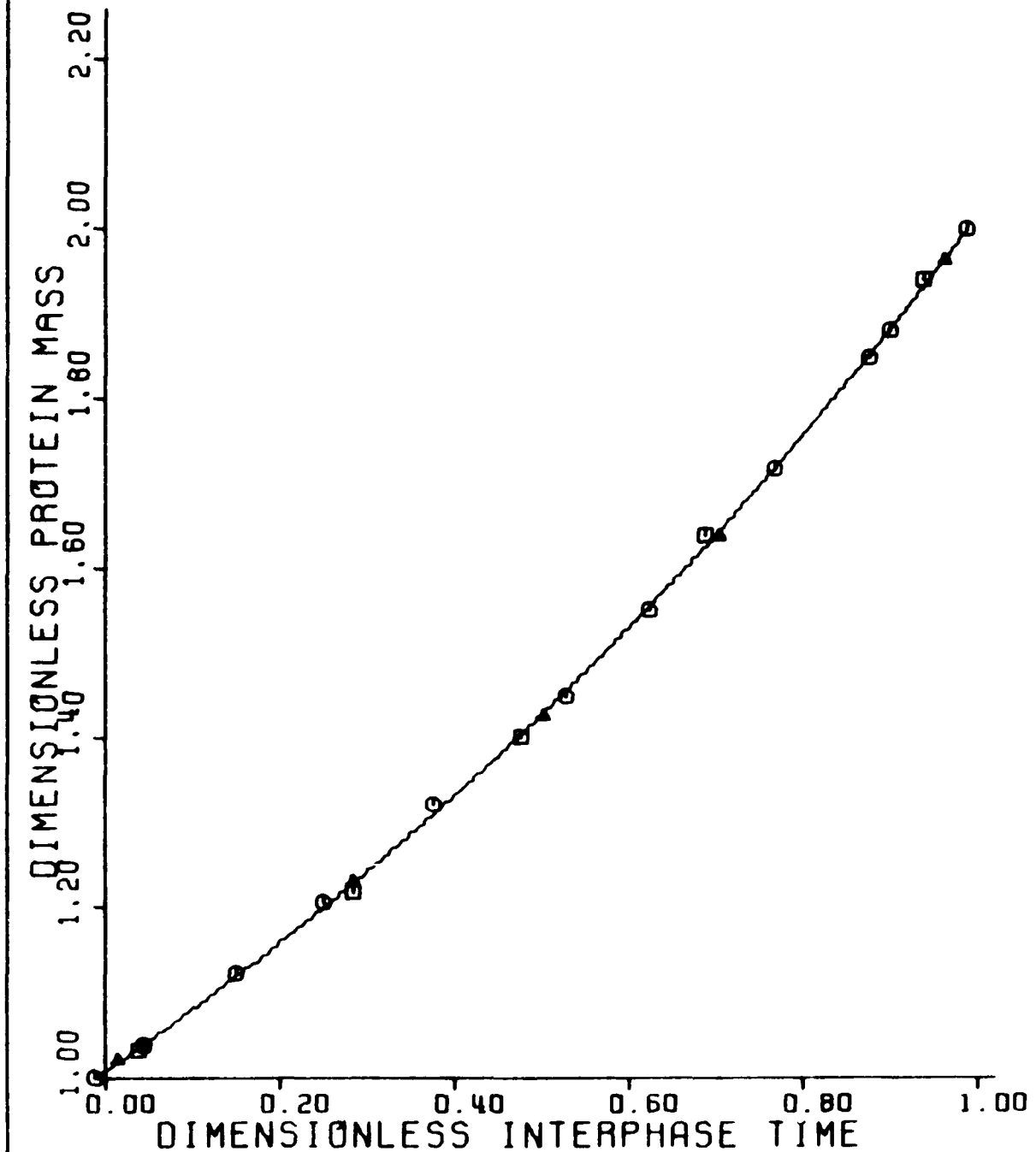
Both the experimental data and the ECM simulation demonstrate that protein synthesis occurs continuously and at a steadily increasing rate. In fact, the rate of synthesis increased over the

# FIGURE III-5

PROTEIN IN MOUSE FIBROBLASTS DURING NORMAL GROWTH

## LEGEND

- EUKARYOTIC CELL MODEL UNDER NORMAL CONDITIONS
- C14 LEUCINE UPTAKE, ZETTERBERG AND KILLANDER (1965)
- ▲ H3 LEUCINE UPTAKE, ZETTERBERG AND KILLANDER (1965)
- S35 METHIONINE UPTAKE, ZETTERBERG AND KILLANDER (1965)



whole of interphase by a factor of approximately 2. This also suggests an indirect gene-dosage effect due to the activity of mRNA in the translation system.

#### 4. Cell Volume

The most complete criterion of growth in cells is volume since it provides a quantitative measure of all the components of living cells. Moreover, volume gives direct information about the increase in the cell membrane or wall and thus is a good measure of the largest constituent of cells - water. Many of the mathematical models of cell growth involve fluctuating concentrations of controlling molecules, therefore volume becomes an essential part of concentration.

A quite sophisticated analysis of volume growth in CHO (Chinese hamster ovary) cells has been performed by Anderson et al. [8]. Sinclair and Ross [9] performed a similar analysis with Chinese hamster cells but, due to relatively poor cell cycle synchronization, their results are not suitable for comparison with the ECM.

Anderson utilized tightly synchronized suspension cultures and mitotic cells from monolayers. Differential cell volume spectra were determined by a Coulter counter technique [10], but it is the mathematical treatment of experimentally determined results that makes his study unique.

A mathematical model was formulated by Anderson in which a given cell was characterized by its age and volume; these parameters were assumed to determine the rate of volume growth. If this method is applied to cultures in balanced, exponential growth, the results

of the Anderson model yield a function,  $f(V)$  (volume growth rate function), which is formulated from terms which are experimentally measurable. One can, therefore, calculate the value of  $f(V)$  for every value of  $V$ , and present data in the form of  $f(V)$  versus  $V$ . Thus, rather than perform the transformation of Anderson's data to obtain volume versus time, the ECM was used to predict  $f(V)$  versus  $V$ . The experimental results of Anderson's four runs are shown together with the ECM simulation results in Figure III-6. The data represents only that portion between the average birth and division volumes from Anderson. Experimental determination of  $G_1$ ,  $S$ , and  $G_2$  times of 4.7, 4.1, and 2.8 hours respectively [11], were utilized as input data to the ECM.

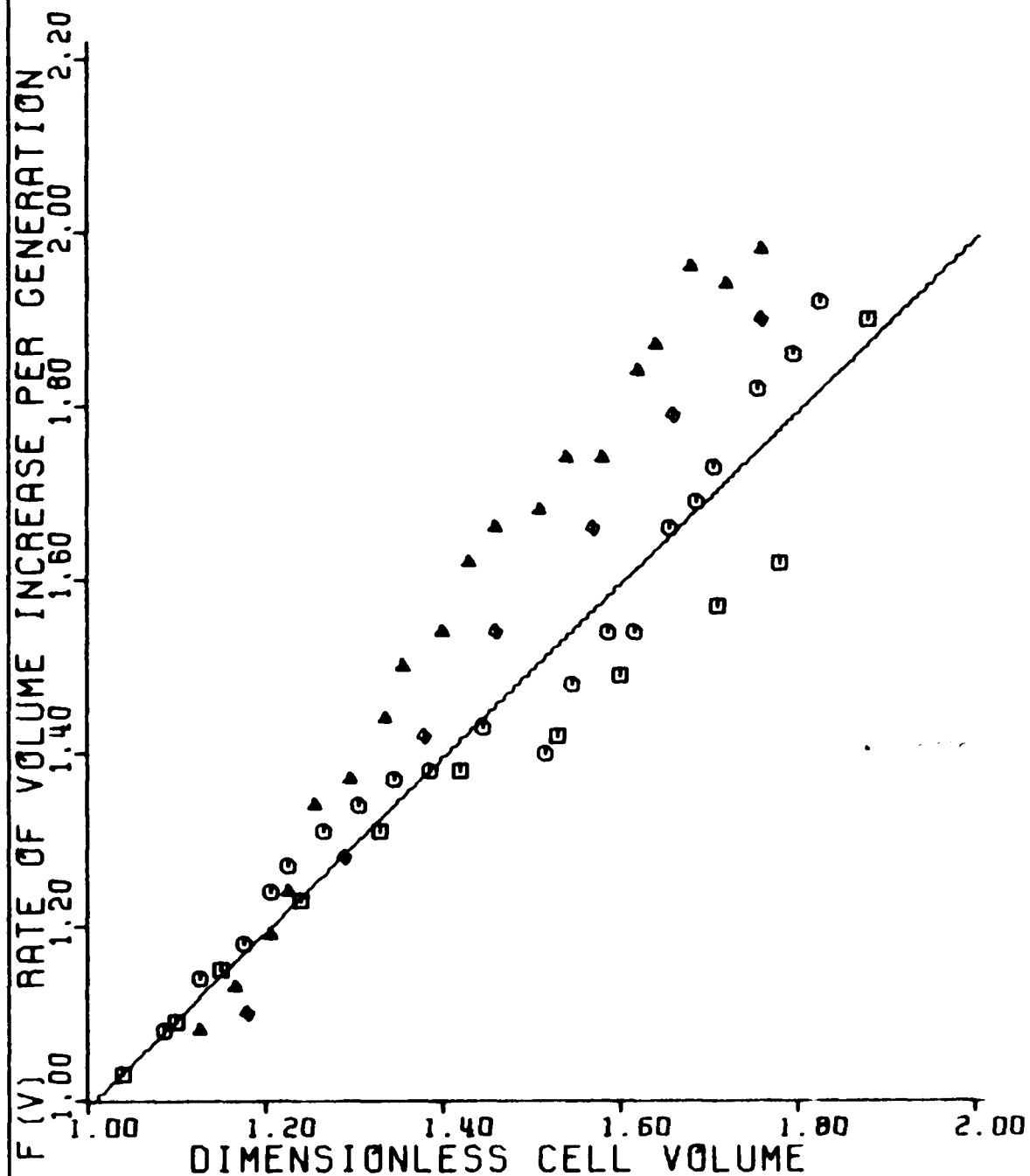
Both the ECM and experimental data indicate that the rate of volume increase is proportional (in this normalized version they are equal) to cell volume at each stage of the cell cycle, thus giving exponential volume growth through the cycle with a doubling in rate from the beginning to the end. It is not surprising to observe an exponential rate of volume increase since this is predictable by simple first-order kinetics. We should anticipate this for the limiting factor for cellular growth in relatively rich media is the total amount of "machinery" (ribosomes, enzymes, etc.) for utilizing substrates. Anderson et al. found that the rate of volume dispersion of the synchronously growing population was very slow. This observed limited rate sets rather severe restrictions on feasible growth laws. In a previous study [12], Bell and Anderson demonstrated that a lack of volume dispersion should be expected for any population of cells

## FIGURE III-6

VOLUME GROWTH RATE IN CHINESE HAMSTER OVARY CELLS DURING NORMAL GROWTH

## LEGEND

- EUKARYOTIC CELL MODEL UNDER NORMAL CONDITIONS
- MONOLAYER AND MITOTIC CELLS, ANDERSON ET AL. (1969)
- ▲ SUSPENSION AND MITOTIC CELLS, ANDERSON ET AL. (1969)
- MONOLAYER AND MITOTIC CELLS, ANDERSON ET AL. (1969)
- ◇ SUSPENSION AND MITOTIC CELLS, ANDERSON ET AL. (1969)



for which the volume growth rate function,  $f(V)$ , is linearly proportional to volume.

In summary, the eukaryotic cell cycle model simulation predicts quantitatively the results of normal growth during the cell cycle in not one but several experiments, with different cells and different G1, S, and G2 growth periods. Profiles consistent with reaction mechanisms were obtained and experimental data from the literature were utilized to support the ECM.

## B. Deviations from Normal Growth

Important in the relationship between mathematical models and the "real world" are validity, generality and prediction utility [13]. The eukaryotic cell model we developed in Chapter II is general in nature and the experimental data from the literature has lent validity to the simulated cell cycle syntheses. The exclusion of some sort of test for predictive ability in this study would be an obvious omission.

### 1. Blockage of DNA Synthesis

Pfeiffer [5] suggested that blocking the initiation of DNA synthesis would, in turn, affect the acceleration of RNA synthesis during the S period in the HeLa S3 cells studied. Due to the nature of the transcription process this most assuredly would occur, but to what degree? Accordingly, cultures of synchronous HeLa cells were exposed by Pfeiffer to hydroxyurea, an inhibitor specific to DNA synthesis, from the time of collection until late in the S period. The cultures were then labeled with  $C^{14}$ -uridine as in the normal growth studies of the preceding section. These cells, along with

untreated control cultures were assayed for the appropriately labeled RNA fractions. To achieve a similar effect with the ECM simulation, DNA synthesis was also "blocked" by changing equation (2-62) from

$$S(\theta) = 1 \quad @ \quad \theta_S \leq \theta \leq \theta_{G2} \quad (2-62)$$

to

$$S(\theta) = 0 \quad @ \quad \theta_S \leq \theta \leq \theta_{G2}. \quad (3-1)$$

This change effectively insures that no DNA synthesis will take place in the ECM. All other conditions are the same as for normal growth. Also, the G1, S, and G2 times as well as the optimal rate constants remain unchanged.

The results of these experiments by Pfeiffer as well as the predicted synthesis patterns from the ECM are shown in Figures III-7, III-8, and III-9 which follow. For comparison purposes, the experimental data and prediction results for the normal untreated control cultures are also depicted. The rate of synthesis for each species of RNA during the normal period of DNA synthesis is much lower than in the untreated control cultures (and model). Pfeiffer's data from cells treated with hydroxyurea and the results of the ECM simulation indicate that only 60-70% as much RNA was synthesized by the treated cultures as by the untreated control cells. This result is expected since the rate of new RNA synthesis, as measured by the uptake of the radioactively-tagged precursor, C<sup>14</sup>-uridine, was modeled to be proportional to the amount of DNA present.

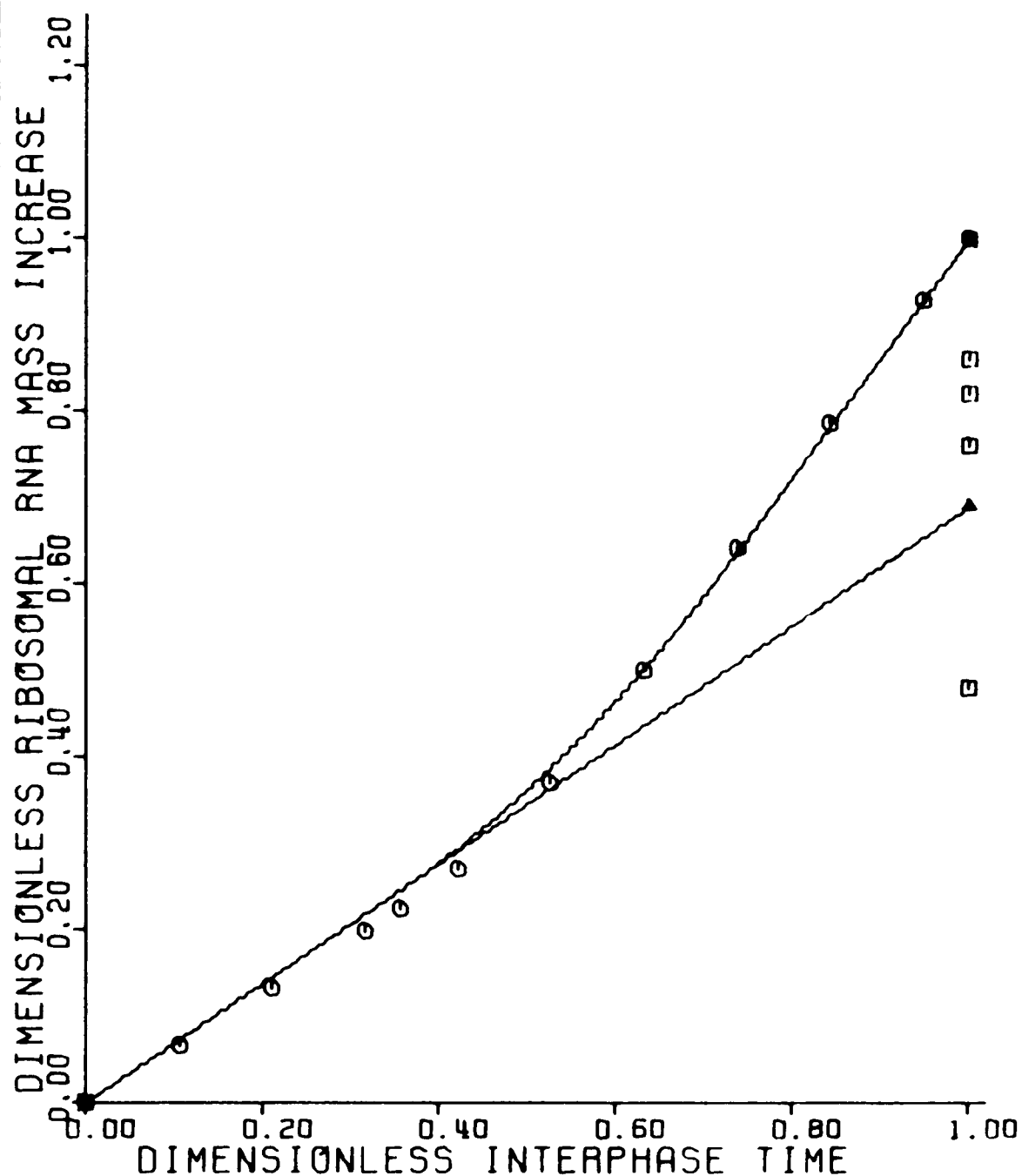


# FIGURE III-7

RIBOSOMAL RNA IN HELA 93 CELLS SUBJECTED TO A BLOCK IN DNA SYNTHESIS

## LEGEND

- X EUKARYOTIC CELL MODEL UNDER NORMAL CONDITIONS
- DATA FROM C<sup>14</sup>-URIDINE INCORPORATION. PFEIFFER (1968)
- ▲ CELL MODEL SUBJECTED TO BLOCK IN DNA SYNTHESIS
- DATA FROM CELLS TREATED WITH HYDROXYUREA. PFEIFFER (1968)

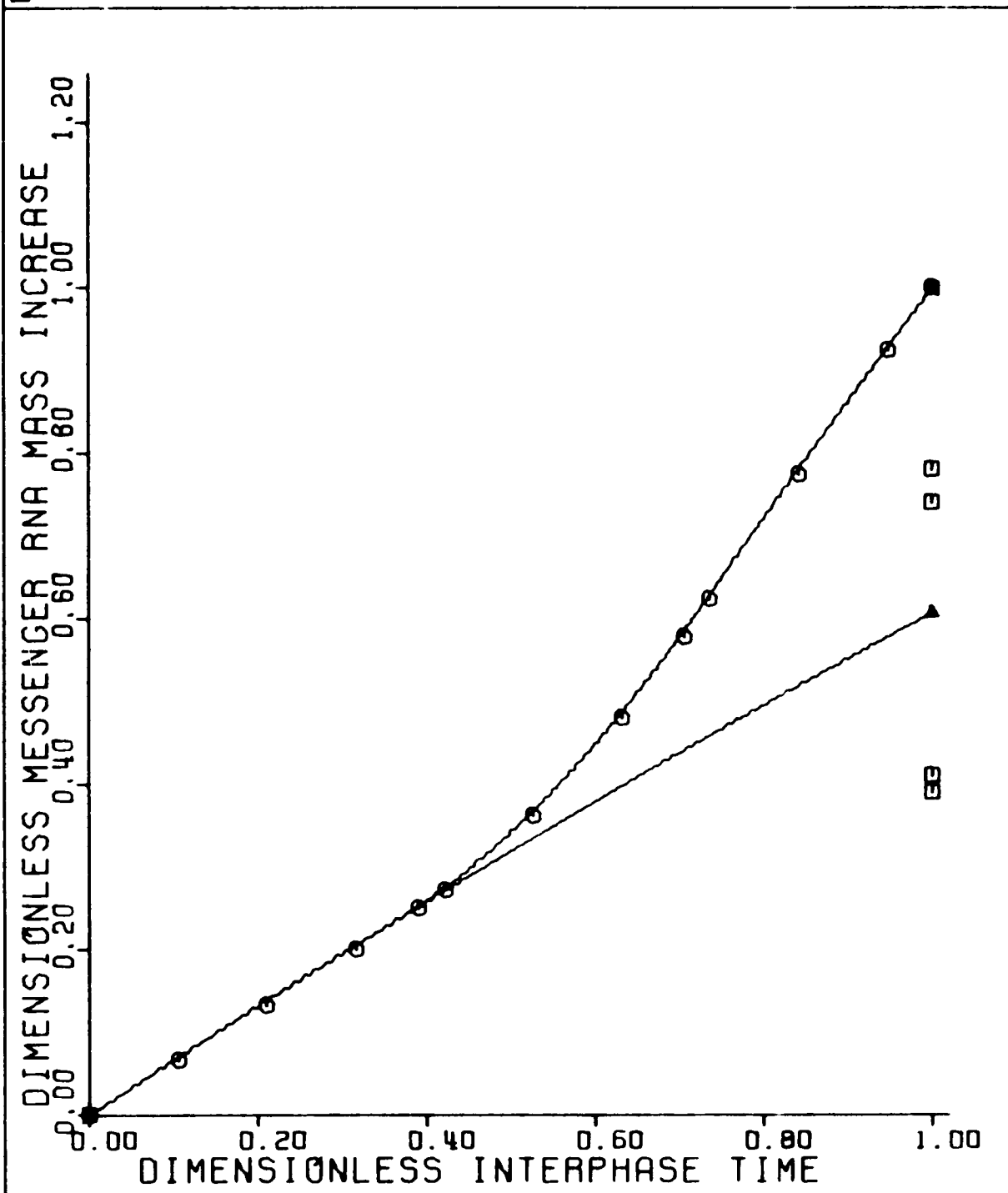


# FIGURE III-8

MESSANGER RNA IN HELA 99 CELLS SUBJECTED TO A BLOCK IN DNA SYNTHESIS

## LEGEND

- X EUKARYOTIC CELL MODEL UNDER NORMAL CONDITIONS
- DATA FROM C14-URIDINE INCORPORATION. PFEIFFER (1968)
- ▲ CELL MODEL SUBJECTED TO BLOCK IN DNA SYNTHESIS
- DATA FROM CELLS TREATED WITH HYDROXYUREA. PFEIFFER (1968)

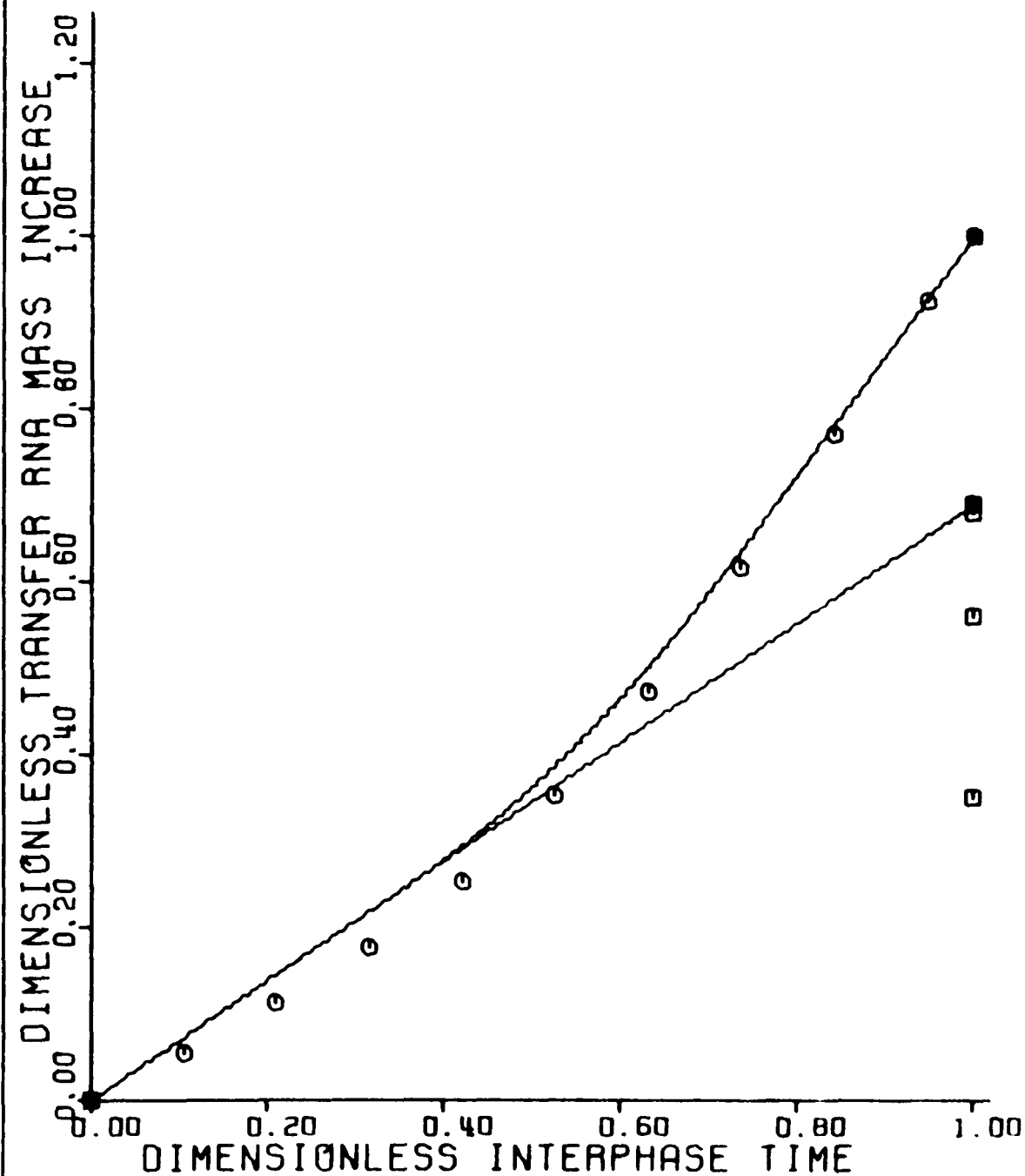


## FIGURE III-9

TRANSFER RNA IN HELA 99 CELLS SUBJECTED TO A BLOCK IN DNA SYNTHESIS

## LEGEND

- X EUKARYOTIC CELL MODEL UNDER NORMAL CONDITIONS  
 O DATA FROM C14-URIDINE INCORPORATION. PFEIFFER (1968)  
 ▲ CELL MODEL SUBJECTED TO BLOCK IN DNA SYNTHESIS  
 □ DATA FROM CELLS TREATED WITH HYDROXYUREA. PFEIFFER (1968)



We have, therefore, in a simplified fashion, developed and tested a general, valid and predictive model of eukaryotic cells. We shall now utilize this model to explore other interesting characteristics of the simplified eukaryotic cell system which it represents.

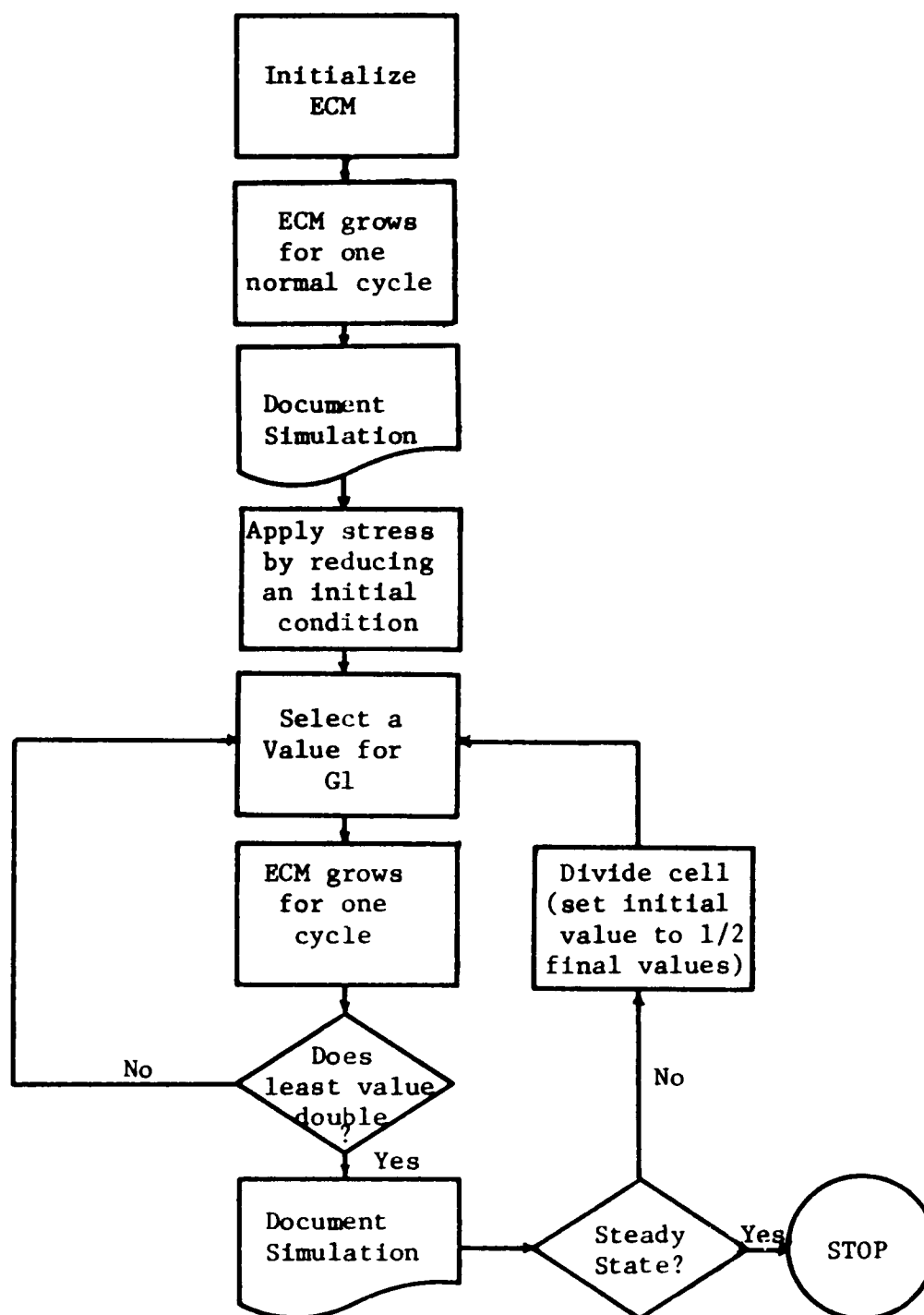
## 2. Stability of Cell Model Subjected to Stress

A great number of biological studies of cells are made in terms of the ability of the cell to form a colony. And, observations taken over a single generation, or cycle, will not necessarily apply to these experiments. It is possible to use the eukaryotic cell model to examine the stability of a cell over several generations, and its reaction to various "stresses" or disturbances from its normal state.

Goodwin [14] has indicated that both the generation time and synthetic patterns of a bacterial cell are normally asymptotically stable; i.e., after most perturbations, a culture of bacteria returns to its original growth conditions. Yeisley and Pollard [1], in their analog computer study of differential equations concerned with bacterial cell synthesis demonstrated that when a stress, such as reducing the original mRNA, or DNA, was applied to the model cell, it either demonstrated asymptotic stability or became unstable depending on the particular "experimental" conditions. Yeisley and Pollard demonstrated the stability characteristics of their bacterial cell model by applying a stress in the fashion mentioned. After starting the simulation they decided that when the component in the smallest quantity doubles its initial value, the cell will

divide. All the other components are now too high. These values are divided by two (cell division), becoming new initial conditions for a second generation of daughter cells. Repeating this sequence of steps, if it proves that the simulation restores itself to the proper initial values after a reasonable number of generations, the cell (culture) is considered to be asymptotically stable. If the series of divisions is clearly divergent, the cell is thus unstable.

In a similar fashion, the eukaryotic cell model simulation is programmed to grow and divide as though it were forming colonies or growing synchronously. The only significant difference in the scheme of analysis used here arises from the basic cell cycle difference between bacterial cells and higher eukaryotes. Eukaryotic cells normally account for variations in growing conditions by adjusting the G1 period and leaving S + G2 relatively constant [15]. In their simulation, Yeisley and Pollard permitted the end of the cell cycle to vary in length, but in this study the G1 period will be allowed to adjust itself from cycle to cycle, to allow the limiting component to double before division, hence S + G2 will be constant. The ECM was allowed to "grow" for one "normal" cycle before the stress was applied. A logical flow diagram for this procedure utilizing the ECM is presented in Figure III-10. In this instance, we are not only interested in finding out if the cell system is stable or not, but we also want to obtain the complete time-dependent solution for the given starting conditions. In addition, the cell model we have developed is nonlinear with discontinuous reaction rates. Due to these considerations, a technique of direct simulation by computer is perhaps the most practical method of attack [16].



Logic Flow Diagram for Stability Study

FIGURE III-10

As examples of the stability characteristics of the ECM to selected stresses, Figures III-11 and III-12 illustrate the time response of free mRNA and protein in mouse fibroblasts (using the model tested for these cells) after a reduction of the initial quantity of free mRNA by 10% of its correct value following one normal cycle. Such a reduction could be physically produced in a eukaryotic cell by the application of an appropriate chemical or radiological treatment. Figures III-13, III-14, and III-15 demonstrate the time response through several cycles of DNA, mRNA, and protein as predicted by the ECM for the same cells after reducing the initial quantity of DNA by 5% of its correct value following one normal generation.

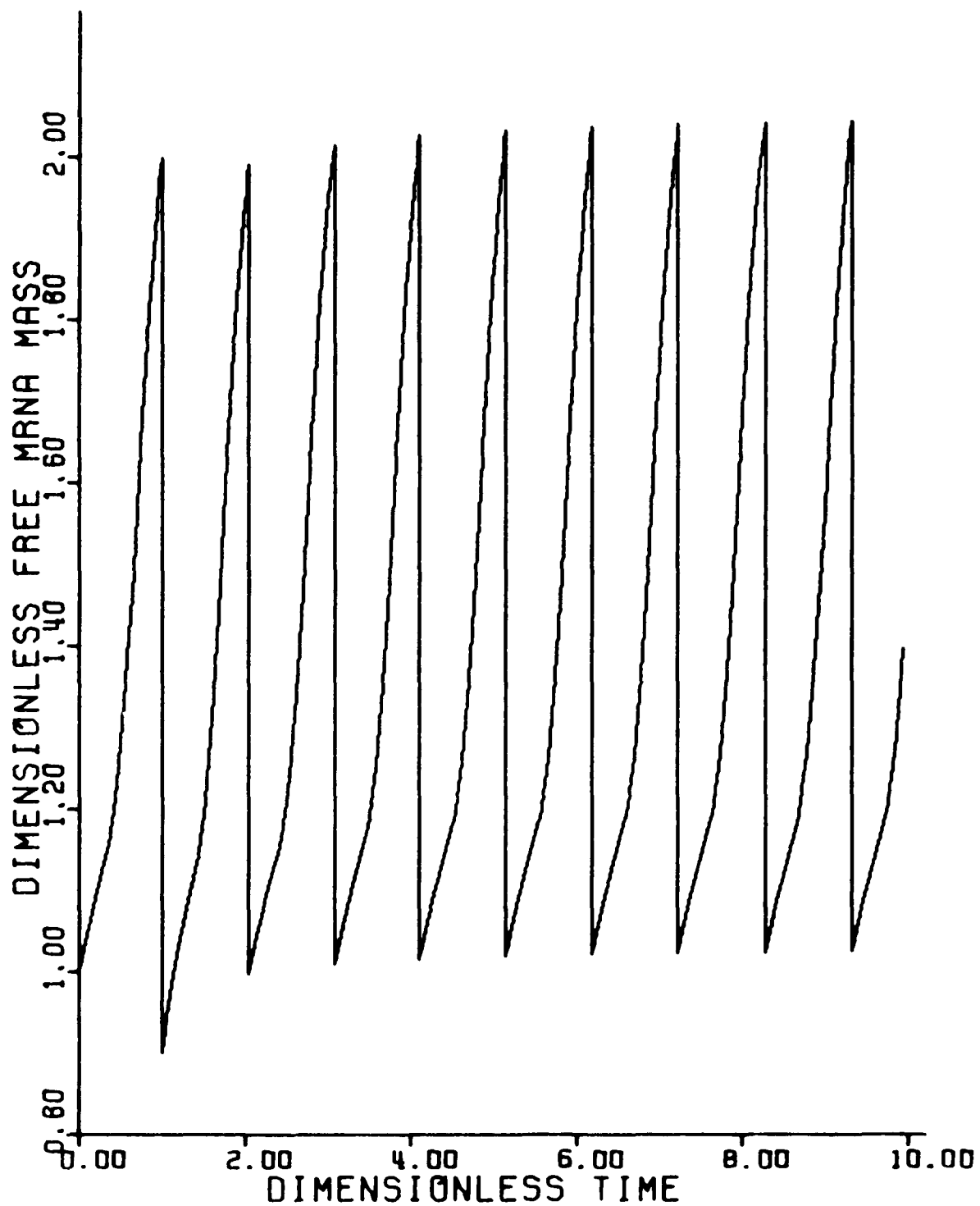
From the simulation figures, we observe that the behavior of our cell model is neither asymptotically stable nor unstable as Yeisley and Pollard obtained from their model of the bacterial cell cycle. The result of stresses in the form of both an mRNA reduction and a DNA reduction demonstrated a non-asymptotic stability described by a return of the ECM system to a new, different cyclic steady state. The application of a chemical or radiological upset, characterized by a step down in RNA or DNA, has caused the cell (culture) to reach a new equilibrium value at which all cell components again double themselves but the relative amounts of each component have changed and the length of the cell cycle has changed. The figures demonstrate that this transient response is stable, not divergent. However, due to a hysteresis effect, there is a permanent offset from the original conditions.

## FIGURE III-11

TIME RESPONSE OF FREE MRNA IN MOUSE FIBROBLASTS TO CHANGE IN FREE MRNA

## LEGEND

- REDUCTION OF 10 PER CENT AFTER ONE NORMAL CYCLE



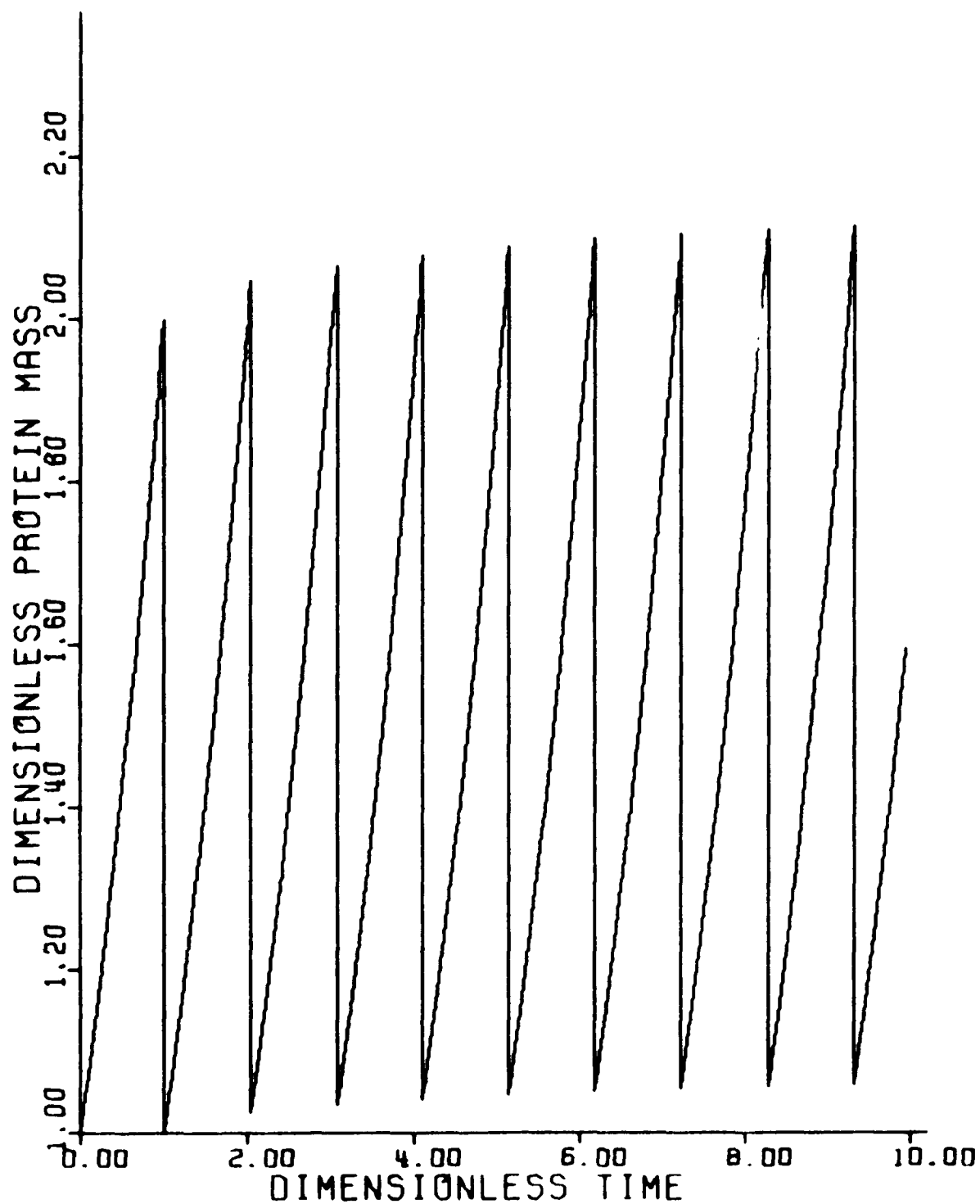


## FIGURE III-12

TIME RESPONSE OF PROTEIN IN MOUSE FIBROBLASTS TO CHANGE IN FREE MANA

## LEGEND

- REDUCTION OF 10 PER CENT AFTER ONE NORMAL CYCLE

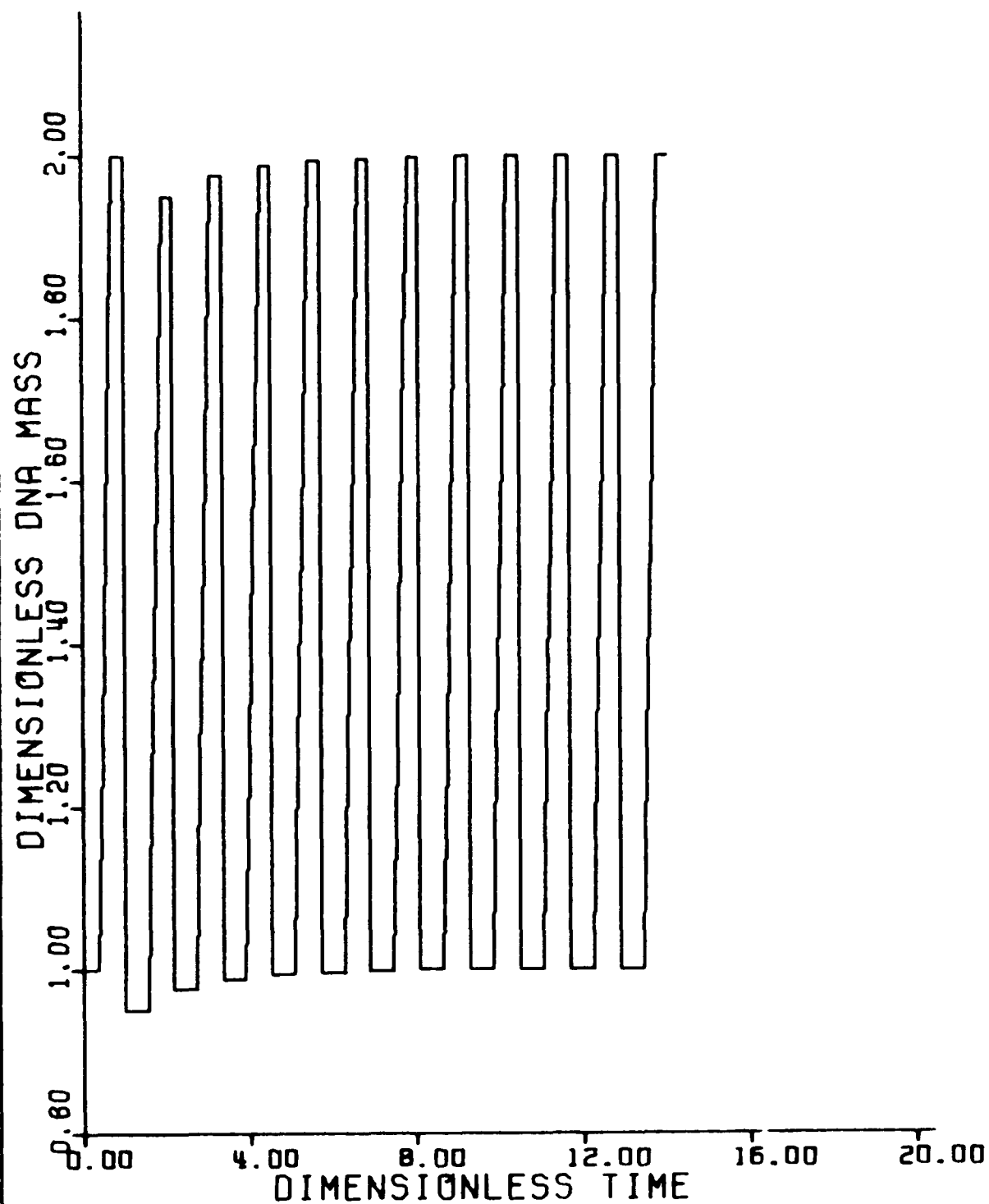


## FIGURE III-13

TIME RESPONSE OF DNA IN MOUSE FIBROBLASTS TO CHANGE IN DNA

## LEGEND

- REDUCTION OF 5 PER CENT AFTER ONE NORMAL CYCLE

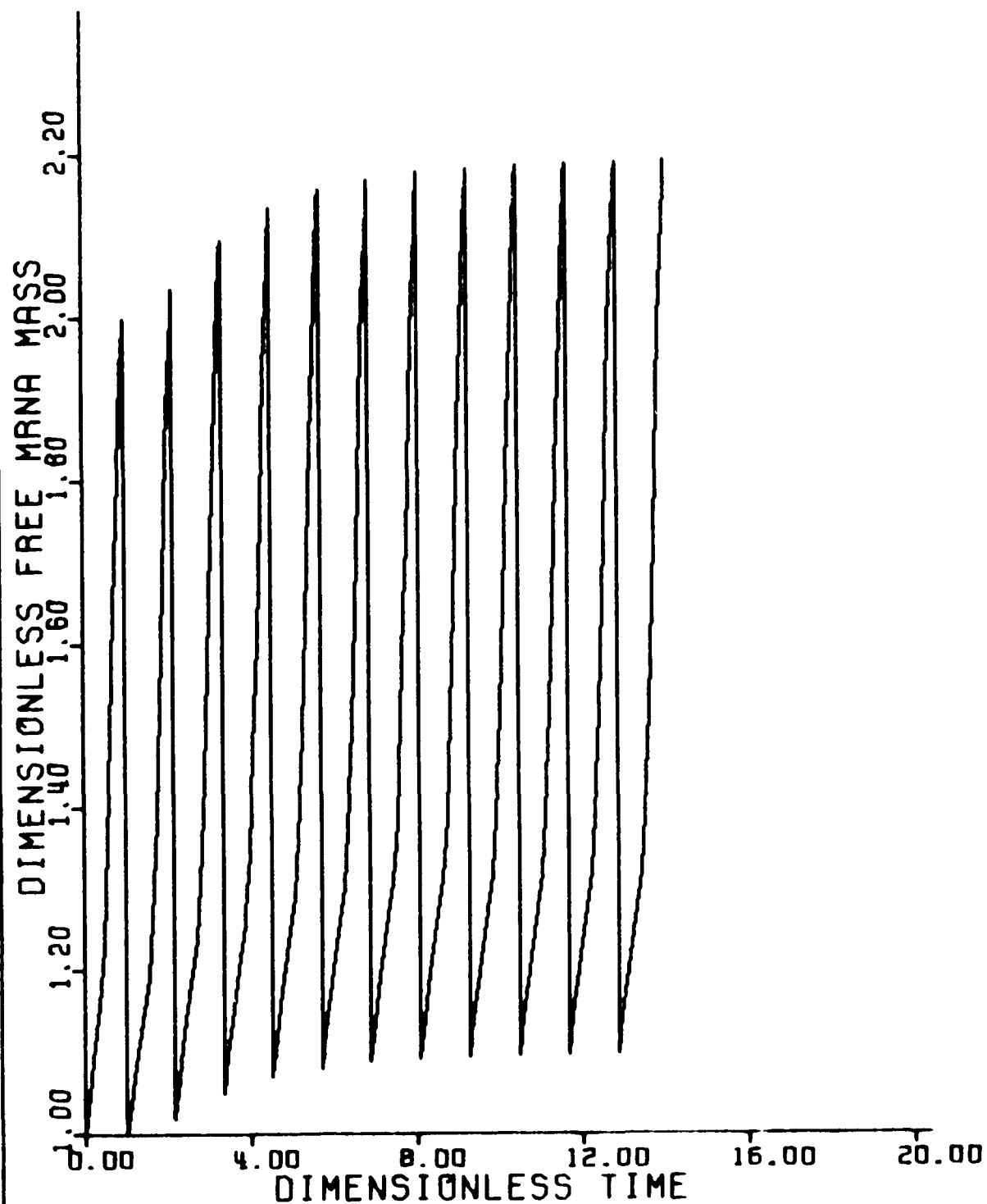


## FIGURE III-14

TIME RESPONSE OF FREE MRNA IN MOUSE FIBROBLASTS TO CHANGE IN DNA

## LEGEND

- REDUCTION OF 5 PER CENT AFTER ONE NORMAL CYCLE

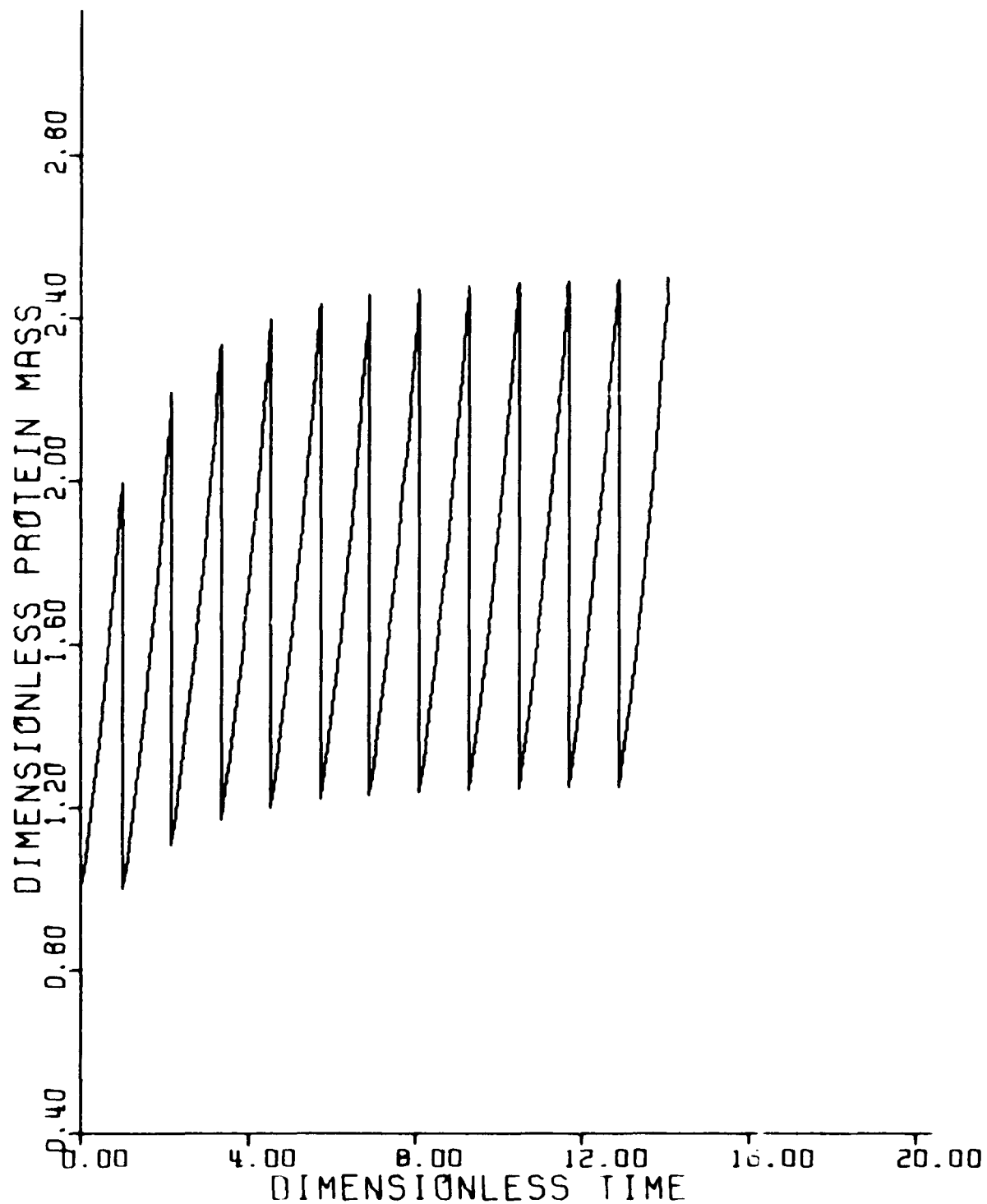


## FIGURE III-15

TIME RESPONSE OF PROTEIN IN MOUSE FIBROBLASTS TO CHANGE IN DNA

## LEGEND

- REDUCTION OF 5 PER CENT AFTER ONE NORMAL CYCLE



These two "stresses" were selected to demonstrate the relative stability of the ECM's response to disturbances of a widely divergent nature. The 5% step down in DNA, Figures III-13 through III-15, is a much more severe test of the ECM, in terms of the deviation from the original cyclic steady state, than the corresponding 10% reduction in free mRNA (as opposed to bound mRNA). This is to be expected considering the closer relationship of the DNA unit to the biological processes.

It was found that the larger the disturbance, the longer it would take to restore equilibrium, although equilibrium would be restored. DNA reductions had the most significant effect on equilibrium shift, but changes in any cell constituent had a measurable effect on displacement from the original steady state.

As to why the ECM predicts a different type of stability from that of Goodwin [14] and Yeisley and Pollard [1], a look now at the cytological evidence available will suggest a possibility.

### 3. Messenger RNA and Cancer

The findings of Yeisley and Pollard [1] suggest that for the bacterial system, at least, the rapid decay of messenger RNA confers a measure of stability to the model system in question. However, if mRNA decay does become slow enough, the bacterial system modeled would eventually become unstable. One of the principal differences between bacterial systems and eukaryotic cells, and thus between the Yeisley-Pollard model and this study, concerns the speed of mRNA decay. The other major difference is the DNA cycle - continuous in bacterial cells and discontinuous in the eukaryotic systems. Yeisley

and Pollard demonstrated for their bacterial model that the introduction of a limited DNA synthesis period did confer some degree of stability on the cell.

What if the ECM were changed only by increasing the speed of decay of the mRNA species involved? In Chapter II, the decay time constant for mRNA breakdown was set at the cell cycle generation time. Both the mathematical model of Yeisley and Pollard [1] and experimental results [17] indicate that the breakdown of bacterial mRNA is some 25 times faster, on the average, than that of eukaryotic cells. Therefore, with

$$k'_5 = 25 , \quad (3-2)$$

we performed the stability simulation in the same manner as in the last study, to observe the effects of fast messenger decay upon cell model stability. For simplicity, only free mRNA was assumed to decay. Figures III-16, III-17, and III-18 illustrate the respective responses of DNA, protein, and free mRNA to a stepdown disturbance of 50% in free mRNA.

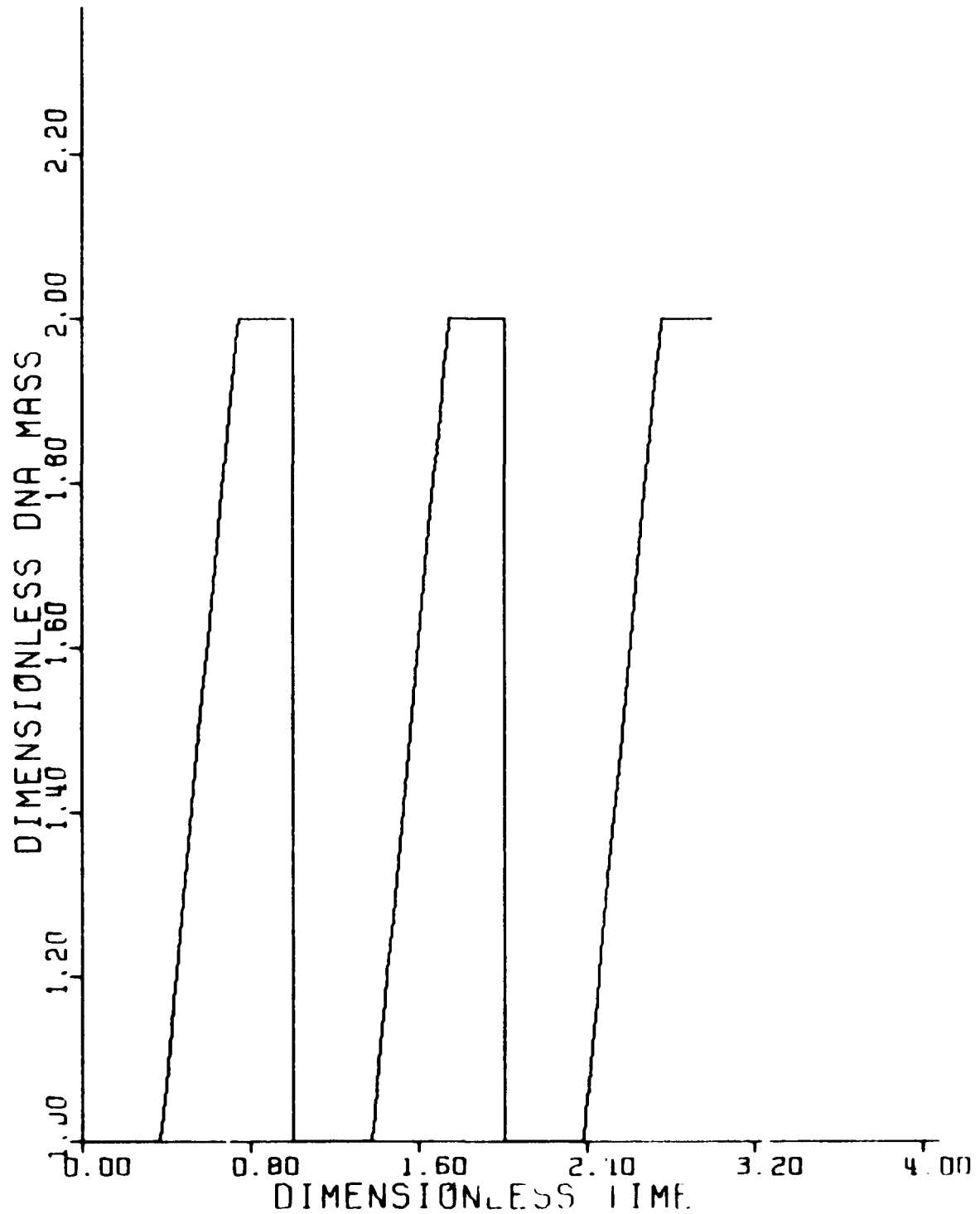
The results of this simulation are dramatically different from that of the normal ECM. In DNA and protein, no measurable effect is discernible; in mRNA, however, after one normal cycle, the disturbances become completely absorbed or damped out. It takes but one cycle for essentially full recovery from an imposed stress that was five times the magnitude of the value applied to the normal ECM. Under normal conditions, the ECM was characterized by an oscillation to a new steady state when subjected to stress. With rapid mRNA decay, asymptotic stability is achieved after only one cycle.

## FIGURE III-16

DNA RESPONSE TO CHANGE IN FREE MANA IN CELLS WITH RAPID DECAY OF MANA

## LEGEND

- REDUCTION OF 50 PER CENT AFTER ONE NORMAL CYCLE

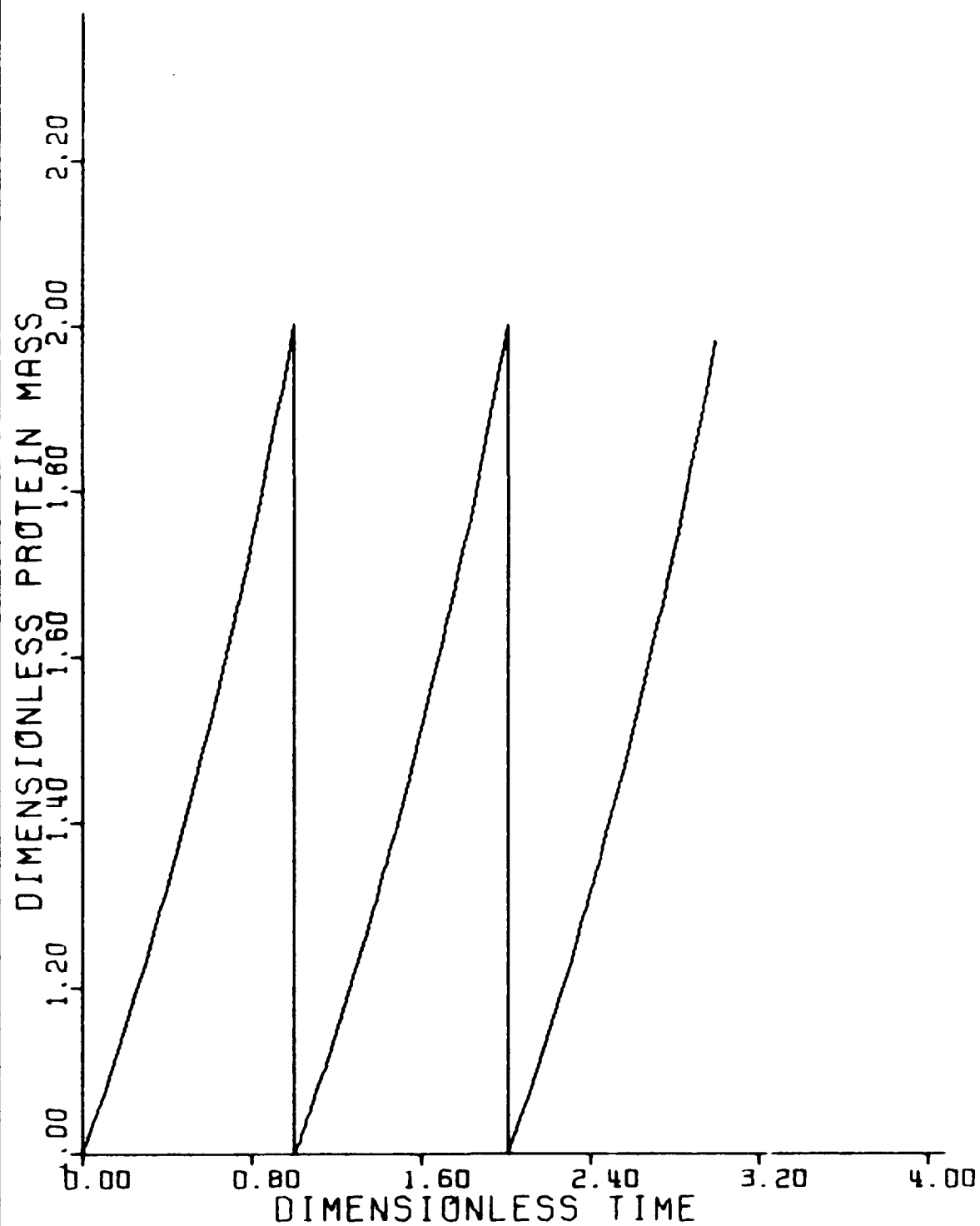


## FIGURE III-17

PROTEIN RESPONSE TO CHANGE IN FREE MANA IN CELLS WITH RAPID DECAY OF MANA

## LEGEND

- REDUCTION OF 50 PER CENT AFTER ONE NORMAL CYCLE



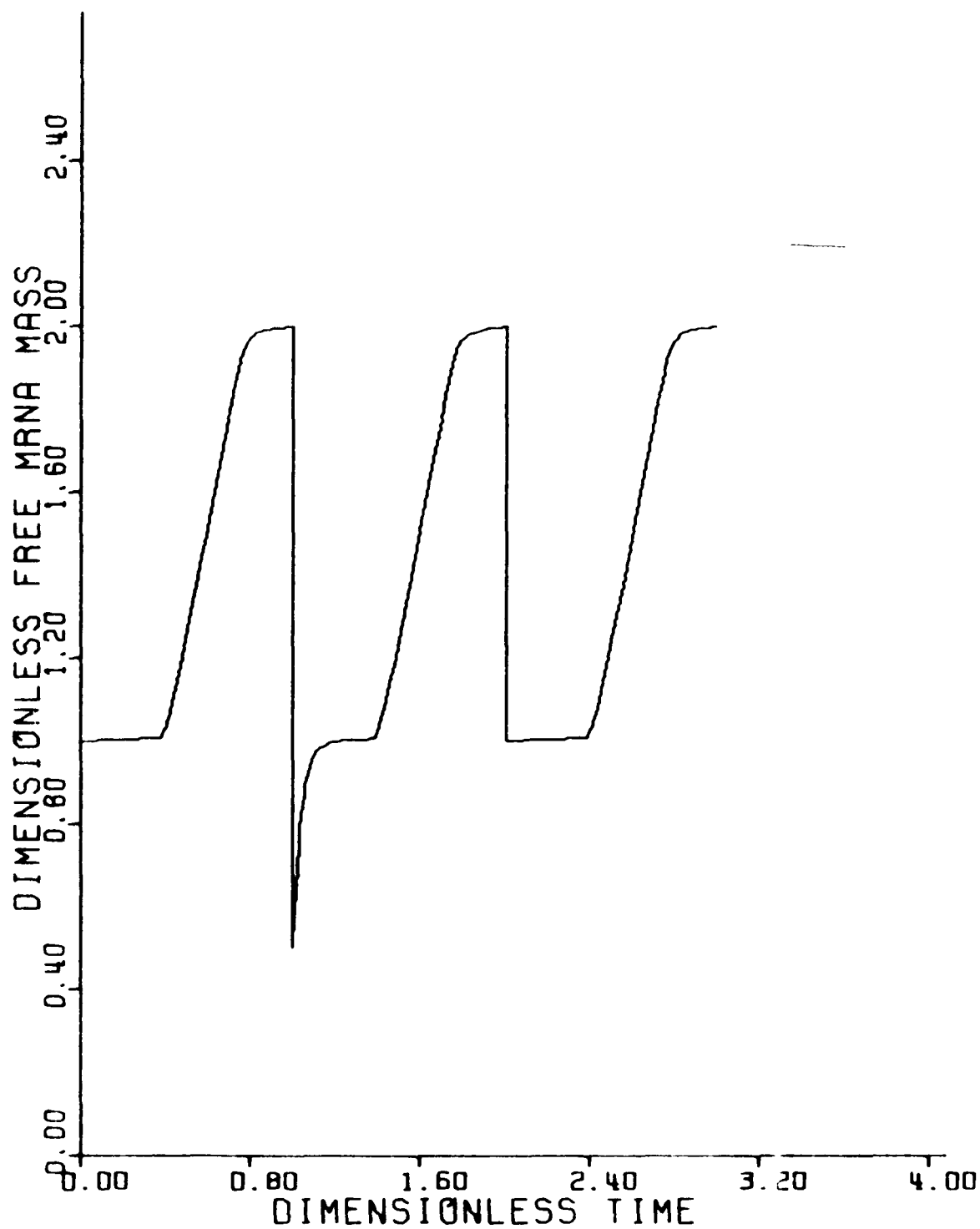


## FIGURE III-18

FREE MRNA RESPONSE TO CHANGE IN FREE MRNA IN CELLS WITH RAPID DECAY OF MRNA

## LEGEND

- REDUCTION OF 50 PER CENT AFTER ONE NORMAL CYCLE



What does this stability analysis mean? And what possible cytological role does mRNA play in eukaryotic cells? These questions and others are not immediately answerable, and only under the most fortuitous circumstances would one expect answers to be forthcoming from the results of a single model study. But impetus for such studies has been supplied by an interest in stability and control processes as they might apply to a topic of more than mere cytological importance -- cancer.

Many concepts of carcinogenesis are based on studies of hereditary concerns and mutation. Pitot and Heidelberger [18] were among the first to disagree with this approach and express the belief that the disease might be caused by a change in cell regulation and not mutation. Gilbert [19] has suggested that carcinogenesis might be due to changes in the absolute and relative dynamic behavior of individual cellular control systems. Finally, Harington [20] went so far as to suggest that carcinogens (cancer forming agents) might function by altering the cyclic patterns of the biosynthesis of one or more products which are specific for normal cell processes. The cell, then, adapts to the disturbance by changing the oscillatory patterns to new, permanent ones; a reaction not unlike that of the ECM. These changes, as part of the cancer processes, are then transmitted by heredity to the progeny of the affected cell.

In none of the previous discussion should it be assumed that the stability characteristics of the ECM under stress represent those of cancerous cells or that the action of messenger RNA decay is the cause for this behavior. But evidence now exists [18, 19, 20]

that the oscillatory patterns of cellular systems in some cases may indeed be related to the action of carcinogens and the resultant cancer process. Perhaps the existence of longer-lived mRNA messages in eukaryotic cell systems does have an effect on the cyclic patterns of synthesis and their displacement to new, permanent levels in response to stress. At any rate, hypotheses resulting from such studies may suggest experiments which will increase the body of evidence in this field.

### C. Initiation of DNA Synthesis

As mentioned in Chapter I, the mechanism for initiation of DNA synthesis is as yet unknown. There is some evidence, however, that in eukaryotic cells there is a relationship between protein synthesis and DNA synthesis. It has been argued that if cells are treated with protein synthesis inhibitors, DNA synthesis either stops or is substantially reduced [21, 22]. In the course of their experiments on mouse fibroblasts, Killander and Zetterberg [2] noted that there was a significantly smaller variation of the dry mass of individual cells at the start of the S period than there was with cells at the start of the cycle. The variation in cell age at the start of S (the length of G1) was also much larger than the mass variation, suggesting that there may be a critical mass at which the cell initiates DNA synthesis. It seems unlikely that mass per se acts as such a signal, but since protein is the principle constituent of cell mass it's very likely that protein could.

In the Killander and Zetterberg study, results were based on individual cells from the same mouse fibroblast cell line. The

normal variation between cells provided the necessary data with which to make statistical inferences. In the ECM, the deterministic nature of the system precludes the existence of any initial variations in cell protein (or mass). The same may be said for cell protein quantity and age at the onset of the S period. However, if the model was used to check the results from various cell types, the variation of cell protein and age at S from one cell type to the next could provide data suitable for statistical inference.

By specifying the ECM for each of the 16 cell types referenced in Table III-1, simulation runs were made and the resulting values for cell protein and cell age (recorded as  $G1/t_g$ ) at the onset of the S period were tabulated. If one hypothesizes that the variation in one of these quantities is statistically smaller than the other ( $p_e < .01$ , where  $p_e$  is the probability that the null hypothesis holds), the latter is considered to be of more consequence in determining the occurrence of the event in question. The results of a statistical study on the relation of protein synthesis to the initiation of DNA synthesis indicate, in Table III-1, that the variation in normalized cell protein is statistically smaller at 9.4%, than the variation in normalized cell age, 34.3%, at the start of the S period. Here we define the coefficient of variation as the ratio of the sample standard deviation to the sample mean.

These results indicate that, on a broader scale, the amount of cell protein appears to be more significant than relative cell age with respect to the initiation of DNA synthesis. Similar studies of an experimental nature would offer conclusive data, but the body of evidence both experimental and theoretical, seems to support the

TABLE III-1

Results of Statistical Study on the Relation of Protein  
Synthesis to the Initiation of DNA Synthesis

Cell Type	Normalized Cell Age G1/t <sub>g</sub>	Normalized ECM Protein P	Reference for G1/t <sub>g</sub>
Human embryonic fibroblasts	0.135	1.099	[23]
Human leucocytes	0.260	1.198	[23]
Human kidney	0.493	1.408	[23]
HeLa	0.589	1.506	[23]
HeLa S3	0.442	1.361	[23]
HeLa S3	0.368	1.293	[5]
Human amnion	0.524	1.439	[23]
Mouse L60	0.550	1.466	[23]
Mouse L60T	0.312	1.243	[23]
Mouse L	0.450	1.368	[23]
Mouse fibroblasts	0.495	1.411	[2]
Mouse fibroblasts	0.405	1.326	[1]
Chinese hamster	0.250	1.190	[23]
Chinese hamster	0.192	1.143	[23]
Chinese hamster	0.562	1.478	[23]
Chinese hamster ovary	0.421	1.341	[10]
Mean	0.403	1.329	
Standard deviation	0.138	0.124	
Coefficient of variation (%)	34.3	9.4	

concept that an "initiator protein", as yet undiscovered, is responsible for the initiation of DNA synthesis in eukaryotic cell systems.

#### D. Summary

In an effort to demonstrate the viability of the mathematical model for higher eukaryotic cell systems derived in this work, simulation studies were performed under both normal and special growth conditions. From this study, we have ascertained that:

- (1) Synthesis profiles, under normal growth conditions, for DNA, RNA fractions, protein and cell volume demonstrated the ECM's validity and generality for this purpose,
- (2) The ECM correctly predicted the response of RNA synthesis patterns under the condition of blocked DNA synthesis,
- (3) The stability characteristics exhibited by the ECM under stress were at least compatible with the belief that alterations in oscillatory patterns in eukaryotic cells could lead to the existence of new, permanent levels - one hypothesis for the mechanism of carcinogenesis,
- (4) The concept of protein-initiated DNA synthesis agrees qualitatively with the ECM's statistical study of several different cell types.

The conclusions and recommendations arising from the results of this study are presented in the following chapter.

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## CHAPTER IV

### CONCLUSIONS AND RECOMMENDATIONS

In relatively simple but general terms, a model capable of simulating the growth and replication processes of higher eukaryotic cells has been developed. This mathematical model incorporated the nonlinear kinetic mechanisms prescribed by the biological processes of replication, transcription and translation. The model was designed to describe the dynamic patterns of synthesis during the cell cycle of typical eukaryotes, growing isothermally in relatively rich media.

A numerical integration scheme based on the Runge-Kutta technique was selected to solve the system of first-order, nonlinear, ordinary differential equations developed from the reaction kinetics. Changes of variable were introduced to add facility and generality to the resulting solutions. A Modified Powell Technique was chosen to form the basis for the multidimensional search necessary to establish a biologically functional model system. A constrained search in the region of physically realistic rate constants indicated the existence of a single, biologically meaningful solution.

Eukaryotic cell models that have been validated by experimental data, have not appeared in the literature. Therefore, the development, solution, and application of such a model to real systems fill a void which currently exists in biological cell simulations. Also, the inclusion of the eukaryotic DNA cycle as well

as volume variability in cell models is not reported in the literature. The author's model accomplishes both these objectives.

An analysis of abnormal growth through simulations of:

- (i) the blockage of DNA synthesis,
- (ii) the imposition of stress, and their effects on cell stability, and
- (iii) the stabilization property of messenger RNA decay

indicated the merit of model study of eukaryotic cell systems. The general model should be reliable for synthesis pattern prediction in cells that aren't markedly different from those described by the model system. The statistical study of some 16 different cell types demonstrated qualitative agreement with experimental results that protein quantity and DNA synthesis initiation in eukaryotes seem related. Indications such as these from model systems can provide directive information for further cytological investigation.

As a result of this research, several possibilities for future work become evident:

a) A more descriptive and realistic modeling of cell division could be developed and incorporated into the present eukaryotic cell model. Metabolites which enter and leave the cell don't really have a uniform concentration throughout the cell, even though an analysis based on such uniformity is a good approximation for many purposes. In a nonspherical cell, distribution of concentrations can cause an unequal distribution of forces which will tend to deform the cell and eventually divide it. In cells of higher organisms, the elaborate mitotic apparatus may be operated by such

volume forces. A distributed model system of a cell utilizing these concepts might well predict division probabilities.

b) The inclusion of cell-cell interaction effects could be studied, for cells growing in tissues are in close proximity to each other and the metabolic and synthetic activities of a cell are surely influenced by adjacent cells. How such interactions lead to cell-cell recognition is of concern to cytologists and physiologists as well. Conceptual models might be developed. The current understanding of cellular processes and entities is sufficient information to begin theorizing, and then modeling the mechanisms of cell-cell interaction and recognition.

c) Cellular differentiation, one of the central problems in developmental biology, could be investigated. It seems that some cells which appear identical, when exposed to the appropriate stimuli, differentiate into different cell types. What is lacking is definite experimental evidence as to when such modifications occur, and how they are triggered. Since we cannot afford to ignore any possible mechanism, genetic or otherwise, mathematical models of this cell phenomenon could well be utilized to explore this, as yet, not fully understood process. Again, as with cell-cell interaction, there is sufficient starting material for a model study of simple differentiation schemes and their incorporation into the basic cell growth model.

In conclusion, investigations into the above areas in conjunction with the more basic studies such as the ECM, will prove valuable in making the transition in understanding from the fundamental unit - the cell, to the ultimate unit - the organism.

## APPENDICES

APPENDIX A  
NOMENCLATURE

<u>Symbol</u>	<u>Description</u>	<u>Units</u>
AR	Moles of activated ribosome	moles
ARAT	Moles of complex for protein synthesis	moles
AT	Moles of complex for protein synthesis	moles
D	Moles of total DNA	moles
$D_{DNA-W}$	Binary diffusivity of DNA and water	$L^2/t$
$D_0$	Moles of initial DNA	moles
$M_{DNA}$	Molecular weight of DNA	M/mole
MR	Moles of mRNA	moles
P	Moles of Protein	moles
R	Radius of spherical cell	L
RR	Moles of rRNA	moles
$S(t)$	Step function for DNA synthesis initiation	none
TR	Moles of tRNA	moles
V	Cell volume	$L^3$
$V_0$	Initial cell volume	$L^3$
X	Moles of decay products	moles
ar	Moles of activated ribosome per initial mole of ribosome	none
arat	Moles of complex for protein synthesis per initial mole of complex	none
at	Moles of aminoacyl tRNA per initial mole of aminoacyl tRNA	none
d	Moles of DNA per initial mole of DNA	none

<u>Symbol</u>	<u>Description</u>	<u>Units</u>
$d_0$	Moles of original DNA per initial mole of DNA	none
$f(V)$	Volume growth rate function	none
$k$	Rate constant for average DNA synthesis	1/t
$k_1$	Rate Constant for DNA synthesis	1/t
$k'_1$	Dimensionless rate constant for DNA synthesis	none
$k_2$	Rate constant for rRNA synthesis	1/t
$k'_2$	Dimensionless rate constant for rRNA synthesis	none
$k_3$	Rate constant for mRNA synthesis	1/t
$k'_3$	Dimensionless rate constant for mRNA synthesis	none
$k_4$	Rate constant for tRNA synthesis	1/t
$k'_4$	Dimensionless rate constant for tRNA synthesis	none
$k_5$	Rate constant for mRNA decay	1/t
$k'_5$	Dimensionless rate constant for mRNA decay	none
$k_6$	Rate constant for activated ribosome synthesis	moles $L^3/t$
$k'_6$	Dimensionless rate constant for activated ribosome synthesis	none
$k_7$	Rate constant for aminoacyl tRNA synthesis	1/t
$k'_7$	Dimensionless constant for aminoacyl tRNA synthesis	none
$k_8$	Rate constant for protein complex synthesis	moles $L^3/t$
$k'_8$	Dimensionless rate constant for protein complex synthesis	none

<u>Symbol</u>	<u>Description</u>	<u>Units</u>
$k_q$	Rate constant for protein synthesis	1/t
$k'_q$	Dimensionless rate constant for protein synthesis	none
$k_{10}$	Rate constant for volume growth	$L^3/\text{mole } t$
$k'_{10}$	Dimensionless rate constant for volume growth	none
$n_i$	Moles of species i	moles
$n_{i_0}$	Initial moles of species i	moles
$p$	Moles of protein per initial mole of protein	none
$p_e$	Probability of error	none
$r$	Distance in radius direction	L
$r_{\text{DNA}}$	Rate of generation of DNA	$\text{moles}/L^3 t$
$r_i$	Rate of reaction for species i	$\text{moles}/L^3 t$
$rr$	Moles of rRNA per initial mole of rRNA	none
$t$	Time	t
$t_{G2}$	Time at which the G2 period starts	t
$t_S$	Time at which the S period starts	t
$t_g$	Generation time	t
$tr$	Moles of tRNA per initial mole of tRNA	none

Greek

$\alpha$	Fourier number for unsteady mass transfer	none
$\Delta$	Change in a variable	none
$\eta_i$	Moles of species i per initial mole of species i	none
$\theta$	Dimensionless time	none

<u>Symbol</u>	<u>Description</u>	<u>Units</u>
$\theta_{G2}$	Dimensionless time at which the G2 period starts	none
$\theta_S$	Dimensionless time at which the S period starts	none
$\theta$	Dimensionless volume	none
$\xi$	Dimensionless radial coordinate	none
$i$	Dimensionless concentration of DNA	none
$^{(i)}\text{DNA}$	Concentration of DNA	moles/L <sup>3</sup>
$^{(i)}\text{DNA}_{(i)}$	Initial concentration of DNA	moles/L <sup>3</sup>
$^{(i)}\text{DNA}_f$	Final concentration of DNA	moles/L <sup>3</sup>

#### Subscripts

e	pertaining to error
f	final condition
i	species i
i	initial condition

#### Superscripts

/	dimensionless quantity
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## APPENDIX B

### GLOSSARY OF CELL BIOLOGY TERMS

Aminoacyl tRNA: Complex consisting of an amino acid and a corresponding tRNA which acts as a carrier to the protein assembly mechanism.

Aminoacyl tRNA synthetases: Enzymes which catalyze the synthesis of aminoacyl tRNA.

Anti-codon: Transfer RNA nucleotide triplet complementary to the messenger RNA codon.

Autoradiography: Technique in which multiplying cells are allowed to incorporate radioactively labeled precursors into certain components after which these components are embedded in photographic emulsion for later examination peculiar to the study involved.

Cell theory: Idea that the cell is the basic unit of life.

Central Dogma: Title given by Crick to the template hypothesis.

Codon: Messenger RNA nucleotide triplet which represents, or codes for, a specific amino acid.

Cofactors: Some requisite cation or anion used to activate an enzyme.

Complementarity: Idea that there exists a distinct relation between structure and function.

Cytoplasm: Mass of clear, semifluid matter surrounding the cell nucleus.

Density gradient centrifugation: An analytical method utilizing high-speed centrifugation of CsCl solution for purposes of separating DNA strands of different densities.

Division period: Time between cell divisions.

DNA: Double helixed-shaped molecule responsible for heredity in any living organism; deoxyribonucleic acid.

DNA polymerase: An enzyme capable of synthesizing DNA in the presence of the necessary precursors,  $Mg^{++}$ , and primer DNA.

Enzyme: Biocatalysts, a class of proteins produced by cells.

Escherichia coli: The common bacterium of the intestinal tract, often used as a benchmark organism in Microbiology.

Eukaryote: The cells of all higher plant and animal forms.

Feulgen stain: A reagent which produces an intensely colored stain when reacted with DNA.

Fibroblast: A type of tissue cell associated with the inflammatory response.

Fixation: Process by which cells are taken out of the growth cycle at a certain point in time for observation.

Gene: The collection of codons responsible for a single polypeptide.

Generation time: The time period elapsing between the moment a cell is born by division of its parent cell and the moment at which it itself divides.

Heavy isotope labeling: The technique of using, say,  $N^{15}$  instead of  $N^{14}$  in a labeled precursor for synthesis mechanisms to be determined by density gradient centrifugation.

Hydroxyurea: A chemical agent noted for its effect of preventing DNA synthesis in certain cells.

Induction synchrony: Technique in which some treatment is applied to an asynchronous culture which makes all the cells synchronous.

Interphase: The time in the cell cycle between division and mitosis.

In vitro: Outside the natural environment of an organism, literally means "in glass".

In vivo: Within the natural environment of an organism, literally means "in the lining".

Mitosis: The cellular process of dividing nuclear material.

Molecular biology: The field of biological science which seeks to explain cellular activity at the molecular level.

Morphology: The study of cell structure.

mRNA: Messenger RNA; that fraction of RNA which carries the codon or genetic information from DNA through to protein.

Nucleic acids: Large polymers made up of complex units called nucleotides.

**Nucleotide:** Molecule composed of phosphoric acid, pentose sugar and either a purine or pyrimidine base.

**Nucleus:** Contains the genetic material, DNA, that determines the specificity of cellular behavior and controls the metabolic activities.

**Polynucleotides:** A polymer of nucleotides joined by phosphate diester bonds between alternating phosphate and sugar groups.

**Replication:** Duplication of the DNA helix in the cell nucleus.

**Ribosomes:** Particles in the cytoplasm of cells made up of RNA and protein with the function of protein synthesis.

**RNA:** Ribonucleic acid; a polynucleotide with various cellular functions.

**rRNA:** Ribosomal RNA; that fraction of cellular RNA which is incorporated into ribosomes.

**Selection synchrony:** Technique of synchronization in which cells at one particular stage of the cell cycle are selected out of a normal asynchronous culture and grown as a separate culture.

**Semi-conservative replication:** Mode of replication in which each of the two new double helices of DNA consist of one chain of the old double helix and a new complimentary chain.

**Stationary phase:** Phase of growth of a culture in which no new growth occurs due to exhaustion of nutrients and/or build-up of toxic products.

**Synchronous culturing:** Growing a culture of cells where the cycle of individual cells have all been brought into phase.

**Template hypothesis:** The idea that RNA which has migrated to the cytoplasm forms a template on which specific proteins are built up.

**Transcription:** The transcribing of a gene's precise nucleotide sequence in a message-messenger RNA.

**Translation:** The translation of the messenger RNA message via transfer RNA and ribosomes, into the form of a synthesized polypeptide.

**Tritiated thymidine treatment:** A method of observing DNA synthesis through use of a radioactive label.

**tRNA:** Transfer RNA; that fraction of RNA responsible for carrying a specific amino acid into the protein synthesis mechanism.

## APPENDIX C

### GLOSSARY OF CELL MODELING TERMS

**Asymptotic stability:** Property of a cell system such that it will return to original steady growth conditions after a disturbance has been applied.

**Automata theory:** Branch of modern mathematics which deals with the formalities of computer programming through the study of devices which operate in a deterministic manner to provide an output that is some logical function of the input.

**Continuous analysis:** The study of system dynamics based on the differential equations of chemical reaction kinetics.

**Cost function:** Function utilized in optimization and search techniques which, when minimized, signifies the satisfaction of the criteria imposed within it.

**Cubic interpolating algorithm:** A third-order formula for approximating a function; for use in finding a minimum approximation in a search routine.

**Descriptive analysis:** Observation and characterization with the purpose of determining structure, organization, and operational characteristics.

**Functional system:** A physically operational cell system which doubles itself during its generation time.

**Instability:** Property of continued unsteady growth after disturbance.

**Logical control theory:** Study of mixing logical switching for certain biological functions with the continuous analysis of chemical reaction kinetics.

**Stability:** Ability of a system to return to steady state after a disturbance.

**Turing machine:** Logical device utilizing a formalized means of writing symbols on tape.

## APPENDIX D

### ORDER OF MAGNITUDE ANALYSIS

This section is included to demonstrate justification for the assumption that, in the eukaryotic cell, concentration or density gradients are negligible. To simplify the analysis, spherical geometry will be used for the cell model.

The existence or non-existence of concentration gradients will be most critical for the largest molecules in the cell system. Therefore, the cell is pictured to consist of DNA and water as an idealization. A differential mass balance can be made for DNA in a binary mixture with water; and since the system is liquid in nature, the overall density can be assumed constant if DNA is in small concentrations (which it is). In a non-convective system, the result of such a mass balance is, in terms of concentration:

$$\frac{\partial \rho_{\text{DNA}}}{\partial t} = D_{\text{DNA-W}} \left\{ \frac{\partial^2 \rho_{\text{DNA}}}{\partial r^2} + \frac{2}{r} \frac{\partial \rho_{\text{DNA}}}{\partial r} \right\} + r_{\text{DNA}} \quad (D-1)$$

where  $\rho_{\text{DNA}}$  is concentration of DNA,

$t$  is time,

$D_{\text{DNA-W}}$  is the binary diffusivity of DNA and water,

$r$  is distance in the  $r$  (radius) direction,

$r_{\text{DNA}}$  is the rate of generation of DNA.

The order of magnitude analysis is properly carried out by first nondimensionalizing the species continuity equation above. The following nondimensional variables are introduced.

$$\rho = \frac{\rho_{\text{DNA}} - \rho_{\text{DNA}_0}}{\rho_{\text{DNA}_0}} \quad (\text{D-2})$$

where  $\rho_{\text{DNA}_0}$  is the initial concentration of DNA,

$$\xi = \frac{r}{R} \quad (\text{D-3})$$

where  $R$  is the radius of the cell,

$$\text{and} \quad \theta = \frac{t}{t_g} \quad (\text{D-4})$$

where  $t_g$  is the cell generation time.

Using the dimensionless variables above, equation (D-1) may be written as

$$\frac{\rho_{\text{DNA}_0}}{t_g} \frac{\partial \rho}{\partial \theta} = \frac{\rho_{\text{DNA}_0} \cdot D_{\text{DNA-W}}}{R^2} \left\{ \frac{\partial^2 \rho}{\partial \xi^2} + \frac{2}{\xi} \frac{\partial \rho}{\partial \xi} \right\} + r_{\text{DNA}} \quad (\text{D-5})$$

The reaction model proposed for DNA in Chapter I is

$$r_{\text{DNA}} = k \rho_{\text{DNA}_0} \quad (\text{D-6})$$

where  $k$  is the reaction rate constant.

The rate of DNA synthesis can also be described as

$$r_{\text{DNA}} = \frac{\rho_{\text{DNA}_f} - \rho_{\text{DNA}_0}}{t_g} \quad (\text{D-7})$$

in an average sense,

where  $\rho_{\text{DNA}_f}$  is the final concentration of DNA.

For the special case where volume is constant and where all components must double during the generation time, one arrives at

$$\rho_{\text{DNA}_f} = 2\rho_{\text{DNA}_0} \quad (\text{D-8})$$

which yields

$$r_{\text{DNA}} = \frac{\rho_{\text{DNA}_0}}{t_g} \quad (\text{D-9})$$

Combining this with (D-5) produces

$$\frac{\rho_{\text{DNA}_0}}{t_g} \frac{\partial \rho}{\partial \theta} = \frac{\rho_{\text{DNA}_0} \cdot D_{\text{DNA-W}}}{R^2} \left\{ \frac{\partial^2 \rho}{\partial \xi^2} + \frac{2}{\xi} \frac{\partial \rho}{\partial \xi} \right\} + \frac{\rho_{\text{DNA}_0}}{t_g} \quad (\text{D-10})$$

Simplifying, rearranging and allowing

$$\alpha = \frac{t_g \cdot D_{\text{DNA-W}}}{R^2} \quad (\text{D-11})$$

$$\text{yields} \quad \frac{\partial \rho}{\partial \theta} = \alpha \left\{ \frac{\partial^2 \rho}{\partial \xi^2} + \frac{2}{\xi} \frac{\partial \rho}{\partial \xi} \right\} + 1 \quad (\text{D-12})$$

Having stated the nondimensional species continuity equation, the problem confronted is estimating the relative magnitude of the terms in the equation.

If  $\rho_{\text{DNA}_0}$ ,  $R$  and  $g$  are considered as the unit orders of magnitude for concentration, distance and time, respectively; the terms  $\rho$ ,  $\xi$ , and  $\theta$  would be of order 1, since each varies from 0 to 1 as a maximum.

Further, since

$$\rho = \int_0^1 \frac{\partial \rho}{\partial \theta} d\theta \quad (D-13)$$

the order of  $\partial \rho / \partial \theta$  can be estimated as 1. That is,  $\rho$  is of order 1 and  $d\theta$  (integrated between 0 and 1) will be of order 1, thus  $\partial \rho / \partial \theta$  must be of order 1 to conserve the order on both sides of the equation:

$$1 = 1 \cdot 1 \quad (D-14)$$

By similar analysis,

$$\text{Order of } \left[ \frac{\partial \rho}{\partial \xi} \right] = 1 \quad (D-15)$$

$$\text{and } \text{Order of } \left[ \frac{\partial^2 \rho}{\partial \xi^2} \right] = 1 \quad (D-16)$$

Thus, the value of  $\alpha$  will decide whether the entirety of equation (D-11) is of order 1 or not. If  $\alpha \ll 1$ , then the second (or diffusion term) can be dropped, as an approximation. If  $\alpha \gg 1$ , the reaction term and time varying term may be dropped. If neither case is true, then all three terms of (D-12) must remain.

Some reasonable values for  $g$  and  $R$  are 20 hours and 10 microns, respectively. A value for  $D_{\text{DNA-W}}$ , however, is much more difficult to identify. Polson<sup>1</sup> finds good agreement, for large anhydrated molecules (molecular weight  $> 1,000$ ) in water at room temperature, with the simple form

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<sup>1</sup>A. Polson, J. Phys. Colloid Chem., 54, 649 (1950).



$$D_{\text{DNA-W}} = 2.74 \times 10^{-5} M_{\text{DNA}}^{-1/3} \quad (\text{D-17})$$

where  $M_{\text{DNA}}$  is molecular weight of DNA

Using a weight of  $5 \times 10^{12}$  for DNA in mammalian cells, the following is obtained:

$$D_{\text{DNA-W}} = 1.6 \times 10^{-9} \text{ cm}^2/\text{sec} \quad (\text{D-18})$$

which sets  $\alpha$ , the Fourier Number for unsteady mass transfer, at

$$\alpha = 100 \gg 1 \quad (\text{D-19})$$

indicating that equation (D-12) can be simplified to the approximate form

$$\alpha \left\{ \frac{\partial^2 \rho}{\partial \xi^2} + \frac{2}{\xi} \frac{\partial \rho}{\partial \xi} \right\} = 0 \quad (\text{D-20})$$

Two integrations using the boundary conditions

$$\rho(0) = \text{finite} \quad (\text{D-21})$$

$$\frac{\partial \rho}{\partial \xi}(1) = 0 \quad (\text{closed wall}) \quad (\text{D-22})$$

yield the result that

$$\rho(\xi) = \text{finite and constant.} \quad (\text{D-23})$$

This analysis, though approximate, indicates that diffusion takes place very rapidly over the small distances involved in cells. DNA was chosen for study as a limiting case. All other cellular

molecules would have given values of  $\alpha$  very much greater than 100, because all other species have much lower molecular weights and therefore higher diffusivities. The assumption of no spatial gradients of concentration appears to be a most tenable one.

## VITA

The author was born in New Orleans, Louisiana on November 25, 1947. He graduated Cum Laude in the Honors Curriculum at Jesuit High School of New Orleans in June, 1965. On January 20, 1968, he married the former Margaret Mary Orkus. The author received his B.S. in Chemical Engineering and his commission as an Army 2nd Lieutenant at Louisiana State University in Baton Rouge in January, 1970. In May, 1971, he received his M.S. degree in Chemical Engineering from the same institution. His daughter, Lauri Angela, was born on August 5, 1971. The author has balanced his academic accomplishments with his musical endeavors as a member of the LSU Marching, Concert and Symphonic Bands. He is presently a candidate for the degree of Doctor of Philosophy in Chemical Engineering.


## EXAMINATION AND THESIS REPORT

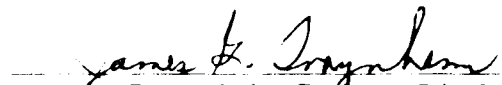
Candidate: Harold Joseph Toups

Major Field: Chemical Engineering



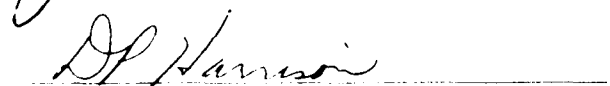


Title of Thesis: A Dynamic Model of the Kinetics of the Cell Cycle  
in Higher Eukaryotes

Approved:

  
Major Professor and Chairman

  
Dean of the Graduate School

### EXAMINING COMMITTEE:

Date of Examination:

July 7, 1973